GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Volume 26 SEPTEMBER, 1941 Number 5

TABLE OF CONTENTS

EMERSON, STERLING, Linkage relationship of two gametophytic characters in *Oenothera organensis* 469

HARMAN, MARY T., and ANNETTE ALSOP CASE, Genetic aspects of pigment production in the guinea pig 474

BRINK, R. A., and D. C. COOPER, Incomplete seed failure as a result of somatoplastic sterility 487

NEEL, JAMES V., A relation between larval nutrition and the frequency of crossing over in the third chromosome of *Drosophila melanogaster* 506

STURTEVANT, A. H., and E. NOVITSKI, The homologies of the chromosome elements in the genus Drosophila 517

MCCLINTOCK, BARBARA, The association of mutants with homozygous deficiencies in *Zea mays* 542

PUBLISHED BIMONTHLY AT MENASHA, WISCONSIN
BY THE BROOKLYN BOTANIC GARDEN
BROOKLYN, NEW YORK, U.S.A.

(Date of issue, September 1, 1941)

EDITORIAL BOARD

M. M. Rhoades, *Managing Editor*, Columbia University

William E. Castle
University of California

Leon J. Cole
University of Wisconsin

Edwin G. Conklin
Princeton University

Charles B. Davenport
Carnegie Institution of Washington

Bradley M. Davis
University of Michigan

L. C. Dunn
Columbia University

Rollins A. Emerson
Cornell University

John W. Gowen
Iowa State College

Herbert S. Jennings
University of California, Los Angeles

Donald F. Jones
Connecticut Agricultural Experiment Station

Thomas H. Morgan
California Institute of Technology

George H. Shull
Princeton University

L. J. Stadler
U. S. Dept. Agriculture
University of Missouri

Alfred H. Sturtevant
California Institute of Technology

Sewall Wright
University of Chicago

C. Stuart Gager, Business Manager, Brooklyn Botanic Garden

Volume 26 SEPTEMBER, 1941 Number 5

Genetics is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $6.00 net a year for complete volumes (January–November). Parts of volumes are to be had only at the single number rate. Foreign postage, 60 cents additional. Single copies, $1.25 each, postpaid.

Back volumes, as available, may be had at $7.00 each, plus postage. The Business Manager will supply information on request as to odd volumes and numbers and complete sets available.

Correspondence concerning editorial matters should be addressed to the Editor of Genetics, 704 Schermerhorn Hall, Columbia University, New York.

Business Correspondence, including change of address, directions concerning reprints, and exchanges, should be addressed to Genetics, Menasha, Wisconsin, U.S.A., or Brooklyn Botanic Garden, 1000 Washington Ave., Brooklyn, N.Y., U.S.A.

Remittances should be made payable to Genetics.

Entered as second-class matter, August 31, 1922, at the post-office at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L. & R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

LINKAGE RELATIONSHIP OF TWO GAMETOPHYTIC CHARACTERS IN OENOTHERA ORGANENSIS

STERLING EMERSON
California Institute of Technology

Received March 8, 1941

IN THE gametophyte generation all genes are normally present in the haploid state, and since the gene effects are not complicated by dominance, it is often possible to apply methods which cannot be used in the sporophyte, or diploid, generation. In this connection, the method used in identifying crossovers between two genes effecting pollen tube growth in *Oenothera organensis* may be of interest.

The growth responses of compatible and incompatible pollen tubes in this species have previously been described (EMERSON 1938, 1939, 1940). The points of importance in the present connection may be briefly recapitulated as follows: Since pollen carrying any one of the 45 known alleles[1] of the self-sterility gene fails to produce normally growing tubes in stigmas or styles of plants carrying the same allele, it is impossible for plants to receive the same allele from both parents. Each plant is thus heterozygous for two alleles, each of which is present in half of the pollen. By pollinating a stigma heterozygous for one of the alleles carried by the pollen, the two sorts of pollen may be readily distinguished. The pollen carrying the allele also present in the stigma is incompatible, producing extremely short pollen tubes (fig. 1B), the pollen carrying the allele not present in the stigma is compatible, producing pollen tubes which continue to grow until they reach the embryo sacs in the ovules (fig. 1A).

The second gametophytic character in this species is a pollen lethal. Pollen carrying this gene, *pl*, fails to produce normally developing tubes in any stigma, regardless of the genetic constitution of the stigma. The lethal pollen tubes are extremely short, much branched and appear to have a much less definite membrane than either compatible or incompatible tubes and so are easily recognized in prepared stigmas (fig. 1C).

OCCURRENCE OF THE POLLEN LETHAL IN THE WILD POPULATION

When allele *20* of the self-sterility series was first found, it behaved normally in that pollen carrying it produced normally developing tubes in all styles not carrying allele *20*. This normal *20* is fairly widely distributed in the Dripping Springs population (EMERSON 1939) where it has been found associated with alleles *5*, *19*, *21*, and *42*. It has also been recovered from open-pollinated seed on a wild plant of the constitution *11*/*19*, indicating that it had been transmitted through the pollen.

[1] The term "allele" is used in this paper at the request of the editor.

GENETICS 26: 469 September 1941. (Date of issue, September 1, 1941)

Second Printing 1963 / University of Texas Printing Division, Austin

In other plants of the same region, allele *20* is associated with the pollen lethal, *20-pl*. These plants reacted in the usual fashion when used as the female parent: pollen carrying alleles other than *20* and the particular second allele present produced normal tubes, whereas pollen carrying either *20* or the particular second allele produced short, incompatible tubes. When

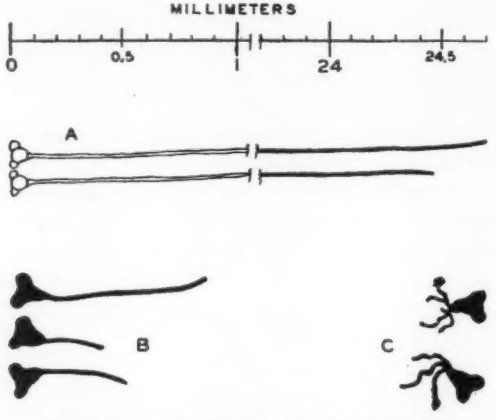


FIGURE 1.—Camera lucida drawings of germinated pollen from prepared styles collected four hours after pollination. A, compatible pollen; B, incompatible pollen; C, lethal pollen.

the same plants were used as the pollen parent, on the other hand, only half of the pollen produced normally growing tubes regardless of the constitution of the female parent, except that on styles having the particular second allele of the pollen parent no normal tubes were produced. These dissimilarities in the results of reciprocal crosses are illustrated by one example in table 1.

TABLE 1

Pollen-tube growth in reciprocal crosses involving the pollen lethal. The + sign indicates that all tubes grew normally (that is, typical compatible tube growth), the − sign indicates that none grew normally, the fraction ½ indicates that only half of the tubes were normal in development.

OTHER PARENT	PARENT *3/20-pl*	
	USED AS FEMALE	USED AS MALE
3/29	½	−
29/32	+	½
20/21	½	½
21/32	+	½

In the plants collected at Dripping Springs, the pollen lethal was found to be associated solely with allele *20*. It was found in four plants occupying

a fairly restricted area (*3/20-pl*, *4/20-pl*, *20-pl/29*, and *20-pl/31*), indicating that the lethal was present for at least one generation before the collections were made. Many other plants of different constitutions from the same canyon were tested as pollen parents and found to be free of the lethal, even though allele *20* was present in some of them.

CROSSING OVER BETWEEN *20* AND *pl*

Open pollinated seed collected from three of the wild plants carrying the pollen lethal gave cultures in which the lethal remained associated with allele *20*, as indicated in table 2. In an attempt to determine if the pollen

TABLE 2

Progeny tests from open-pollinated seeds of plants carrying the pollen lethal.

FEMALE PARENT	RECOVERED ALLELE	WITH LETHAL	WITHOUT LETHAL
3/20-pl	*3*	0	4
	20	1	0
20-pl/29	*20*	11	0
	29	0	18
20-pl/31	*20*	5	0
	31	0	4

lethal ever separates from allele *20*, crosses of the type *3/4*×*3/20-pl* were made and the styles examined for normally growing tubes. In such crosses, pollen carrying allele *3* fails to produce normal tubes because it is incompatible with the tissues of the stigma, but pollen carrying allele *20* is compatible and should produce normal tubes whenever it becomes dissociated from the pollen lethal. Data from experiments of this type together with those from compatible and incompatible controls are summarized in figure 2.

Not all compatible tubes grow at the same rate, and some incompatible tubes may make an appreciable growth. Consequently in the cross *3/4* ×*3/20-pl* it is not always possible to distinguish between compatible tubes (*20* without *pl*) and occasional long, incompatible tubes (*3*). The incompatible controls in figure 2 give an indication of the frequencies of long, incompatible tubes to be expected. If these frequencies are subtracted from the frequencies of tubes of corresponding lengths in the cross *3/4*×*3/20-pl*, the remainder represents the frequency of compatible tubes (*20*) of the same lengths. Since some compatible tubes are known to be relatively short, this value should be increased by a factor derived from the compatible controls in order to obtain the frequency of compatible tubes among all those carrying allele *20*. Such calculations based on tubes of various

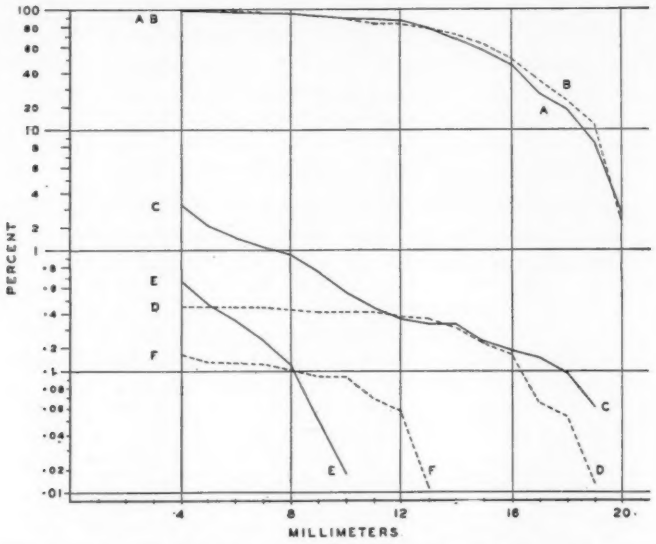


FIGURE 2.—Frequencies of all pollen tubes of a given length or longer in various crosses, four and one-half hours after pollination. Curve A, *3/4×12/13*, all tubes compatible, N=140; curve B, *3/35×12/13*, all tubes compatible, N=165; curve C, *3/4×3/20-pl*, N=8250 (that is, for the number of tubes carrying allele *20* there are also an equal number of tubes carrying allele *3*); curve D, *3/35×3/20-pl*, N=7650 (as in C); curve E, *3/4*×self, all tubes incompatible, N=5900; curve F, *3/35*×self, all tubes incompatible, N=8700. The total number of germinated pollen grains was estimated and is subject to an error of something like ten percent.

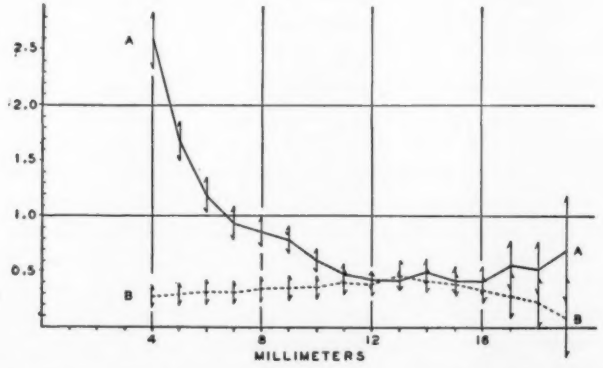


FIGURE 3.—Frequencies, in percent, of recombinations between allele *20* and the pollen lethal based on the frequencies of pollen tubes of different minimum lengths (see fig. 2 and text). Curve A, from pollinations made on *3/4* stigmas; Curve B, from pollinations on *3/35* stigmas. The vertical lines represent plus or minus the standard errors.

minimum lengths are summarized graphically in figure 3. Calculations based on tubes of relatively short minimum lengths (the left-most sections of the curves in fig. 3) are necessarily inaccurate because of the irregular reactions of incompatible pollen tubes. Otherwise the data are in fair agreement with a recombination value of between 0.3 and 0.5 percent between allele *20* and the pollen lethal.

The long pollen tubes in the crosses *3/4×3/20-pl* and *3/35×3/20-pl* were assumed to carry allele *20* without the pollen lethal because from the many thousand tested incompatible tubes (unpublished data) no mutations at the self-sterility locus have occurred. This point was checked by pollinating stigmas of *3/35* with 800 to 1000 pollen grains from *3/20-pl* flowers, observing the seed set and testing the resulting plants for the self-sterility alleles carried. The results of these tests are summarized in table 3 and are in agreement with the interpretation and with the frequency of recombination between *20* and *pl* expected from the pollen-tube behavior reported above.

TABLE 3

Tests of seeds arising from recombinations between allele 20 and the pollen lethal.

CROSS	NUMBER OF FLOWERS	NUMBER OF POLLEN GRAINS	NUMBER OF SEEDS	RECOMBINATION FREQUENCY	CONSTITUTION OF PROGENY	
					3/20	*20/35*
3/35×3/20-pl	18	14,400–18,000	25	approx. 0.3	1	7
3/35×self	18	14,400–18,000	0			

It is assumed that the appearance of allele *20* without the pollen lethal in the instances just reported is the result of crossing over between the two genes, but the loss of the lethal might just as well be due to mutation of the lethal itself. The first alternative could be proved only by obtaining the lethal with the second allele of the self-sterility gene involved (*3* in these instances) which would require the raising and testing of at least a thousand plants from crosses such as *3/20-pl×3/4*, and this has not been feasible.

LITERATURE CITED

EMERSON, STERLING, 1938 The genetics of self-incompatibility in *Oenothera organensis*. Genetics **23**: 190–202.

1939 A preliminary survey of the *Oenothera organensis* population. Genetics **24**: 524–537.

1940 Growth of incompatible pollen tubes in *Oenothera organensis*. Bot. Gaz. **101**: 890–911.

GENETIC ASPECTS OF PIGMENT PRODUCTION IN THE GUINEA PIG[1]

MARY T. HARMAN AND ANNETTE ALSOP CASE[2]
Kansas State College, Manhattan, Kansas

Received March 14, 1941

INTRODUCTION AND REVIEW OF LITERATURE

THE purpose of the present study was to correlate the processes of pigment formation as observed in fetal and adult guinea pigs with the theory of color inheritance as presented by BOGART and IBSEN (1937). The effects of some of the color-detemining factors of the guinea pig and the time and manner of their action have been described.

The foundations of the present knowledge of color inheritance in the guinea pig were laid by CASTLE and WRIGHT (1916), WRIGHT (1917, 1925, 1927), and others. WRIGHT (1917) advanced an enzyme theory of pigment production. In 1927 he presented this theory again in modified form and reviewed the literature on pigment production.

MAXIMOW and BLOOM (1934) summarized from a histological point of view the status of knowledge of pigment and its production in the skin and hair of man. GORTNER (1911) studied the chemical nature of the melanins. EDWARDS and DUNTLEY (1939) found a diffuse pigment in close association with granular pigment in the living human skin. GREMMEL (1939) reported that the coat color of horses was due to different arrangements and concentrations of one kind of granular pigment. He observed no diffuse pigment in horse hairs.

ESSKUCHEN (1927, 1930) described the development of pigment in cattle fetuses. He found two types of pigment cells in the skin; observed that dark color appeared earlier than red and that the skin of the extremities showed pigmentation first.

The relation of pigmentation to color inheritance in pigeons was investigated by HAWKINS (1931).

HUNT and WRIGHT (1918) made a number of microscopic observations on the hair of guinea pigs. They described black and "yellowish" granules and a yellowish diffuse pigment in sectioned hairs.

BOGART and IBSEN (1937) examined the hair and in some cases the skin of various domestic animals, particularly cattle, in an attempt to determine the effects of some color genes upon the pigmentation of the hair and skin.

[1] Contribution No. 217, Department of Zoology, KANSAS STATE COLLEGE OF AGRICULTURE AND APPLIED SCIENCE.

[2] The writers are indebted to DR. H. L. IBSEN and RALPH BOGART, who contributed valuable advice and criticism from the geneticist's point of view; and to LEE FENT, who prepared the photographs. DR. IBSEN selected the animals which were used; the KANSAS ACADEMY OF SCIENCE granted a part of the funds for the maintenance of the colony.

In guinea pigs, they found both granular and diffuse types of pigment in the hair. They distinguished between black and chocolate granules and found that variations in the shade of the diffuse pigment, as well as in the amount and kind of granular pigment, were responsible for the different shades of red hairs. These results formed the immediate basis of the present study.

MATERIAL AND METHODS

Guinea pigs of known genetic composition with regard to the factors studied were bred under observation, and fetuses were obtained which ranged from 43 to 57 days, copulation age. A few newborn animals were also studied. Blocks of skin were taken from the nose, ears, back, and belly of each fetus and adult animal killed. These tissues were studied microscopically as fresh smears and as fixed and sectioned material. A variety of fixatives were used, including 8 percent neutral formalin and 95 percent alcohol, neither of which altered the color of the pigment. Most of these tissues were sectioned and mounted without staining. Bouin's fluid was used as a fixative for tissues which were stained with hematoxylin and eosin and studied histologically. Whole mounts were made of hair samples from animals of various ages.

By these methods the effects of the following color-determining genetic factors were studied: The extension series: E, e^p, and e; Black and chocolate: B and b; The "albino" series: C, c^d, c^r, and c^a. Material was not available for a study of the factor c^k in the "albino" series.

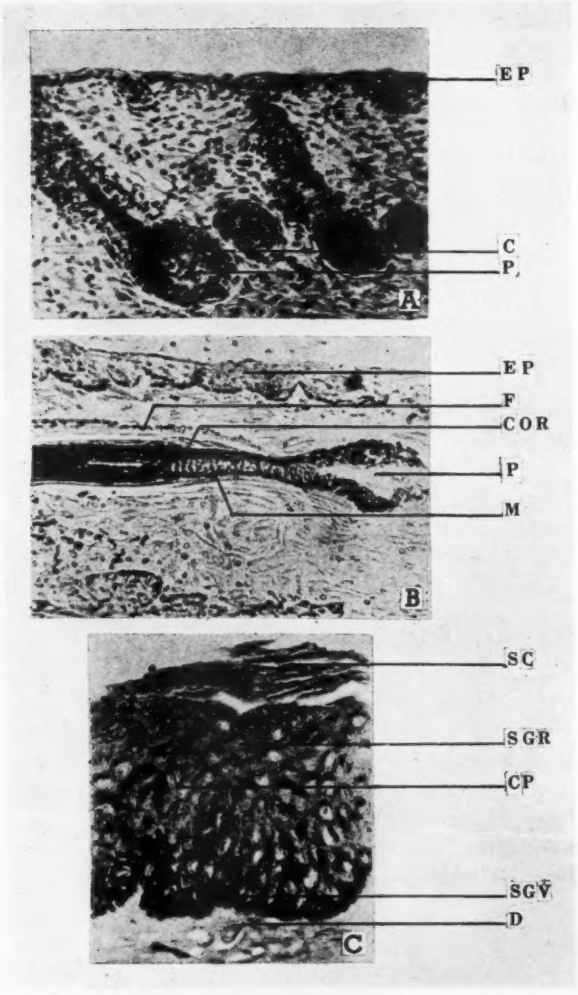
The appearance of the living animals at various ages was checked with the microscopic observations. Color in animals is influenced by non-genetic as well as by genetic factors; therefore, the food, light, and temperature in the colony were kept as constant and as favorable as possible, and the animals used for breeding were not subjected to any experimental treatment.

In the following discussion, the age of embryos is given in days calculated from the date of copulation; other specimens are described either as newborn (less then one week old) or as adult (more than four weeks old).

TYPES OF PIGMENT

Two types of pigment—granular and diffuse—were observed. Granular pigment was of three kinds—red, black, and chocolate, found in red, black, and chocolate hairs, respectively. Black and chocolate granules only were seen in the skin. Colorless granules were found in the white hairs of c^r and c^a animals. They occupied a position corresponding to that of the colored granules in pigmented hairs. In every instance, the color of the hair was the same as that of the granules which it contained.

Diffuse pigment ranged in shade from a pale yellow to a deep red-brown.



EXPLANATION OF PLATE I

FIGURE A.—Transverse section (×148) through the skin of the back of a self intense chocolate fetus, aged 45 days. Fixed in Bouin's fluid, stained with hematoxylin and eosin. The general histological relations of the developing skin and hair are shown. EP, epidermis; C, cone of pigment cells; P, papilla of the hair.

FIGURE B.—Transverse section (×156) through the tip of the ear of a self intense chocolate animal. Formalin-fixed, unstained. The distribution of pigment in the hair (seen in longitudinal

It was seen only in the cortex of the fully formed hair and was always found in association with red, black, or chocolate pigment granules.

DEVELOPMENT OF PIGMENT

The earliest stages of pigment formation were observed in fetuses of 43 days copulation age. Cells containing pigment granules were seen in the anlage of the hair, which was at this time a slight condensation of ectodermal cells projecting into the dermis. At the same time similar pigment cells were observed in the deep layer of the developing epidermis.

These pigment cells were larger than the ordinary cells of the dermis and epidermis and usually were irregularly star-shaped, with long pseudopodia-like processes which extended far out between the surrounding cells. Mitotic figures were often seen in them. Cells of this type were found both in the epidermis and in the hair, but none occurred in the dermis, nor in the dermal hair papilla.

At a later stage of development (45 and 46 days) the branched pigment cells in the base of the follicle were arranged in a dense conical cap over the hair papilla (fig. A). They were pushed upward by the division and growth of the cells next to the surface of the papilla, and they became differentiated into two types: the polygonal cells of the medulla and the spindle-shaped cells of the cortex of the hair. Branched pigment cells also occurred in the external root sheath of the follicle, especially in black and chocolate animals (fig. B).

The definitive hair was composed of a large medulla made up of star-shaped cells between which lay anastomosing air spaces; a rather thin cortex made up of flattened spindle-shaped cells; and a cuticle of thin overlapping scales (fig. B). The medulla was narrow and discontinuous toward the distal end of the hair and was absent at the tip.

The epidermis of the body was thin, and in pigmented animals branched pigment cells and scattered pigment granules were present in its deep layer. The epidermis of the nose and the ears became greatly thickened and contained, in addition to the branched type of pigment cells, several layers of small rounded cells in which granular pigment was found (fig. C).

Pigment granules were seen in the cytoplasm of both types of pigment cells. They were either scattered or gathered into caplike masses over the peripheral surface of the nucleus in the small rounded cells of the skin of

---

section) and in the skin are typical. EP, epidermis; F, pigment in the wall of the hair follicle; COR, cortex of the hair; M, medulla of hair; P, papilla.

FIGURE C.—Transverse section (×443) through the skin of the ear of a self intense black animal. Formalin-fixed, unstained. The stratum corneum is pulled away from the stratum granulosum. SC, stratum corneum; SGR, stratum granulosum; CP, "cap-like" mass of pigment granules at peripheral end of cell; SGV, stratum germinativum; D, dermis.

the nose and ear. The flat cells of the stratum corneum contained fewer granules than the cells in the deep layer. In the branched type of pigment cell the granules were scattered through the cytoplasm or clumped in one or more rounded masses which were often so compact that the individual granules could not be distinguished. Either masses of granules or scattered granules or both could be found in one cell. Pigment granules were also present between the cells of the hair bulb. Cytological observations indicated that the pigment did not originate directly from the nucleus but was formed in the cytoplasm of the cell.

The time and place of development were the same for black, chocolate, and red granules.

Study of the distribution of pigment granules in the hair was complicated by the presence of refractive surfaces in the medulla which appeared dark by transmitted light and often looked like pigment. The following methods were therefore employed in an effort to avoid confusion of pigment with "air spaces":

(1) comparison of cleared, uncleared, and partially cleared hairs;
(2) examination of these hairs with reflected as well as transmitted light;
(3) examination of sectioned hairs.

The results show that while pigment is present in the medulla of many hairs, air spaces are always present and may look dark by transmitted light. It is possible that in some cases where pigment has been described in "albino" hairs, the air spaces might have been mistaken for pigment granules.

DESCRIPTION OF PHENOTYPES

Intense Black (*C- E- B- P-*)

The granular pigment of black animals was dark sepia in color. Rows and clumps of the granules were abundant in the cortex of black hairs, and "black" granules were densely packed in the shrunken cells of the medulla. A faint yellowish diffuse color was visible in the cortex of the hair. This diffuse pigment was somewhat darker at the tip of the hair and was almost absent at the base. "Black" granules were found in the pigment cells of the epidermis and of the wall of the follicle.

Intense Chocolate (*C- E- bb P-*)

Chocolate granules were more translucent and were of a lighter brown color than the "black" granules. The amount and distribution of granules in the hair and the skin of chocolate animals was the same as it was in black animals. The pigment cells were the same as to size, shape, and distribution in both types. Diffuse pigment in the cortex of a chocolate hair

was more intensely red and more conspicuous than it was in a black hair. Granular pigment in the skin was of the same color as that in the hair.

Black and chocolate granules appeared to be the same with regard to size, number, and distribution, and the time of their appearance. In the earlier stages of development, when only a few granules were present in the skin and hair, it was difficult to distinguish between black and chocolate granules. A difference in color was apparent only when a number of granules were observed together. Chocolate pigment appeared to be more susceptible than black to the effects of dilution factors such as c^r and p.

Deep Cherry Red (*C- ee B- P-*), (*C- ee bb P-*)

All cherry red hairs which were studied contained translucent bright red-orange granules, abundant in the medulla and rather sparsely scattered in the cortex. A brilliant orange-red diffuse pigment was also present in the cortex. It was brighter near the tip of the hair than at the base. No black or chocolate granules were seen in any red hair, whether in a spot on an e^p animal or on a self red. Red granules were larger than black and chocolate granules, and they were more irregular in shape. Their color was entirely different from that of black or chocolate; no intergrades were found.

Red granules from different animals were sometimes different in shade; this variation seemed to be due in part to differences in the age of animals and in part to the influence of the factors *B* and *b*.

Sections of the skin of a spotted red-and-chocolate fetus were particularly valuable for comparing red and chocolate pigments. It was possible to observe, in one field of the microscope, follicles which were developing red hairs and follicles which were developing chocolate hairs. The differences could be observed easily and could not be ascribed to variations in the method of handling or preparation, since both were in the same tissue and were viewed under the same conditions of light and magnification. Red pigment in a spot on such a specimen appeared the same as that in a self red individual.

From observation of fresh whole mounts of the skin of black and red fetuses (litter mates) it was determined that the skin pigment is the same in both *B-* red and black animals. The branched pigment cells of the skin contained black granular pigment in both fetuses, although the hairs of the red fetus contained red granules and those of the black fetus contained black granules.

The skin of the nose and ear of *B-* red animals contained black granular pigment, although there was a little less present in red than in black animals. Chocolate granules were present in the same locations in *bb* red animals.

Newborn deep cherry red animals were a much deeper red and were more brownish than adults of the same genetic composition. This agrees with the observation that in the earlier stages of their development black, chocolate, and red granules look alike. However, microscopical observations showed that hairs from these young red animals were more like reds with very concentrated pigment than like chocolates or blacks.

Light Red ($c^d c^d$ *ee bb smsm P-*)

Hairs from animals of this composition had a few pale red granules in the cortex and the medulla. There was a small amount of yellowish diffuse pigment present in the cortex. It could be seen only at the tip of the hair. No chocolate granules were seen in the hair.

c^r *Black and White* ($c^r c^r$ e^p- *B- P-*)
c^r *Chocolate and White* ($c^r c^r e^p$- *bb P-*)

Some of the c^r black animals were heterozygous for *B;* hence they produced both c^r black and c^r chocolate fetuses, which could not be distinguished from each other at an early age. When the difference could be recognized, as was possible in older fetuses, it was the same as the difference between *C-* black and *C-* chocolate, which has already been described. For these reasons, c^r black and c^r chocolate animals are described together.

To gross observation, c^r black and c^r chocolate hairs appeared lighter in shade, especially near the base, than *C-* black and *C-* chocolate hairs. Microscopical examination showed that c^r hairs had fewer pigment granules both in the medulla and cortex than *C-* hairs but that the granules themselves were typically chocolate or black.

Red diffuse pigment was found in the cortex of c^r chocolate and c^r black hairs. It was less conspicuous in black than in chocolate hairs.

White hairs from the c^r spotted animals contained neither red nor dark granules nor diffuse pigment; however, colorless granules were present in them. (The term "dark" is used here as it was by WRIGHT (1917) to include both black and chocolate granules, hairs, or animals, as distinguished from red or white.) The difference between white and dark hairs was especially evident in sections of the skin of these e^p (spotted) animals, which showed chocolate, white, and "mixed" hairs in the same field of the microscope. In the skin, scattered dark pigment granules were often present in regions where the hair was white as well as where it was pigmented.

c^r White ($c^r c^r$ *ee bb P-*) ($c^r c^r$ *ee B- P-*)
"Albino" White ($c^a c^a$ *ee bb P-*)

No difference was observed among the above three types of white hair. (*E- bb* and *E-B-* "albinos" were not available for study.) Neither red nor

dark pigment granules, nor diffuse pigment, were found in any of the white hairs which were studied. Colorless granules were seen in both c^r and c^a white hairs. They occupied a position in the cell and in the tissue comparable to that of the pigment granules in pigmented hairs.

DISCUSSION

Development of Pigment

In those tissues of the guinea pig examined in this study the pigment was confined to the epidermis and its derivatives. No pigment cells were seen in the dermis nor in the dermal papilla of the hair.

When the anlage of the hair first appeared, pigment cells could be identified in it. At the same time, similar pigment cells could be seen in the developing epidermis around the hair. The time of appearance of pigment was the same in all specimens studied, regardless of the genetic constitution. Hairs in several stages of development could be found on different body regions of a single fetus. It was possible to observe corresponding degrees of pigmentation in them.

These observations are in contrast with the findings of ESSKUCHEN (1927, 1930), who reported that the time at which pigmentation appeared in cattle fetuses varied with the color. Reddish-brown pigmentation appeared three and a half months later than black pigmentation. ESSKUCHEN (1927) also stated that in cattle the extremities were the first to show pigmented areas. The same was found by the present writers to be true in guinea pigs. In some types of guinea pigs, such as c^r whites and some albinos, the extremities are the only places where pigment ever appears.

ACTION OF GENETIC FACTORS

The following observations agree with those of BOGART and IBSEN (1937): (1) Pigment in the hair of guinea pigs occurs in two forms, granular and diffuse. (2) The granular pigment is black in black hairs and chocolate in chocolate hairs. (3) Diffuse pigment occurs as several different shades of red.

The observations given below disagree with the conclusions of BOGART and IBSEN (1937): (4) Red hairs contain red granular pigment, but no black or chocolate pigment. (5) White hairs from all animals studied may contain colorless granules, but no red, black, or chocolate granules. (6) Diffuse pigment is seen only in the cortex of the fully formed hair, and it is associated with some type of granular pigment. The intensity of the diffuse pigment is related to the type of granular pigment with which it is associated. (7) Diffuse pigment occurs in c^r dark hairs, as well as in C and c^d hairs. None is found in white hairs.

BOGART and IBSEN (1937) described the diffuse red pigment in guinea

pig hairs as if it alone accounted for the color of red hairs. They thought that in dark hairs the black or chocolate granules were "extended" so that they obscured the red diffuse pigment and that in red hairs, the black or chocolate granules were present but were not extended, thus permitting the red diffuse pigment to show through. They thought that a complete suppression of red diffuse pigment and the restriction of black or chocolate granular pigment to the medulla accounted for the colorless appearance of white hairs.

We found neither black nor chocolate pigment in *ee* (red and white) hairs. Red hairs contained red granules and white hairs contained colorless granules; however, the two may not be homologous. The red granules are probably comparable to the "yellowish" granules which HUNT and WRIGHT (1918) described in red and yellow guinea pig hairs. HAWKINS (1931) stated that the red coloring in the feathers of pigeons was due to red granular pigment.

Action of Factors in the Extension Series

On the basis of the above observations, it seems that the factors *E* ("extension") and *e* ("non-extension") should be described as determining the production of a definite type of granular pigment (dark or light), rather than the extension or non-extension of pigment granules over diffuse pigment. The light pigment produced in *ee* animals would then include the various shades of red granules, and possibly colorless granules. The dark pigment produced in *E*- animals would then include black and chocolate granules. The distribution of pigment in the hairs from the dark and light areas of e^p animals is the same as that in the hairs from *E*- and *ee* animals, respectively.

Action of Factors in the "Albino" Series

The factors in the "albino" series are discussed here in terms of their relation to the factors *E* and *e*.

In *C*- ("dark eyed") animals, the factor *E* determines the presence of dark granules in the hair. The factor *e* determines the presence of red granules in comparable positions. The action in the skin is probably quantitative rather than qualitative, for no red granules were seen in the skin of any type of guinea pig studied.

Too little work was done with c^d animals to give a basis for describing the effects of that factor.

In c^r ("red eyed," "non-red") animals, the factor *E* produces dark granules in the hair, but *e* does not produce red granules. Instead, it may produce the colorless granules which were observed. Dark granular pigment appears in the skin of c^r white animals, especially in the extremities.

The factor c^a ("albino") is epistatic to both *E* and *e*. Neither red nor

dark granules were seen in the hair of $c^a c^a$ animals of our stock; but colorless granules were seen there as they were in c^r animals. Here again, the action is not the same in the skin as it is in the hair. "Albino" guinea pigs usually have dark pigment in the skin of the extremities. Because of the presence of this pigment, $c^a c^a$ guinea pigs are not considered true albinos.

The Relation Between Diffuse and Granular Pigment

When the actions of the factors in the "extension" and the "albino" series are interpreted as above, it becomes evident that the diffuse red color need not be considered as one of the pigments which is primarily determined by the genetic composition of the animal. The granular pigments seem to be the direct expression of the action of the color-determining factors. The color of the hair in each case corresponds to the color of the granules in it. The red diffuse pigment appears to be formed in the cortex of the hair as a consequence of the presence there of some type of granular pigment. It behaves as if it were the product of a chemical or physical reaction between the granules and the cornified substance of the cortex of the hair. GORTNER (1911) in his description of two kinds of melanin says:

"Those melanins which are soluble in dilute acids are of a protein nature (melanoproteins). It appears probable that these melanoproteins are not present as granules, but that they are 'dissolved' in the keratin structure.

"The melanins which are insoluble in dilute acids are of an unknown constitution and are probably the 'pigment granules' which may be seen in the hair and tissues."

EDWARDS and DUNTLEY (1939), in studies of the living human skin, described a modified diffuse form of melanin which they called "melanoid." It resembled melanin closely in its spectrophotographic properties, and it occurred only in cells derived from melanin-bearing cells—that is, the cells of the stratum corneum. The amount of melanoid in any skin depended upon the amount of melanin found there. Because of these relations, the authors regarded "melanoid" as a degradation product of melanin. It was present as a dissolved pigment and reenforced the melanin, particularly in giving a purer yellow color than melanin alone.

The relations described by EDWARDS and DUNTLEY (1939) for the human skin are similar to those found in the hair of the guinea pig. These authors did not study the hair of their subjects.

In c^r guinea pigs, white hairs are found in place of the red hairs of *C*-animals. BOGART and IBSEN (1937) assumed that these hairs were white, because they lacked red diffuse pigment. They expected that c^r black and chocolate hairs would also lack diffuse red pigment. The presence of red diffuse pigment in c^r black and chocolate hairs at first seemed contradictory to the description of the c^r factor ("non-red"). Its presence may be

explained if one assumes that it is red granular pigment rather than red diffuse pigment, which is determined by the *C-* factor in the presence of *ee* and which will not appear in $c^r ee$ hairs. In the absence of these red granules no red diffuse pigment is formed; hence $c^r ee$ hairs are white. In $c^r E$- hairs the diffuse pigment is formed as it is in *C-E-* hairs, in association with the black or chocolate granules which are present in the hair. The close association between granular and diffuse pigment is parallel to the description of the "albino" series as given by BOGART and IBSEN (1937), in which there is no separate factor for the red diffuse pigment.

The absence of all pigment from the hairs of "albino" ($c^a c^a$) animals may be explained similarly. In such animals neither red nor dark granules are present in the hair; hence no diffuse pigment is produced, and the hair is white.

Action of the Factors B (black) and b (chocolate)

The differences between black and chocolate pigment granules as seen in *E* animals have been described earlier in this paper. Part of the difference between the types of granules seems to lie in their relation to the diffuse type of pigment. Red granules are bright and translucent and are associated with a large amount of diffuse pigment. Chocolate granules are darker and denser, and less of the diffuse pigment is found with them. Black granules are darker than chocolate granules and are associated with an even smaller amount of diffuse pigment.

When deep cherry red (*ee*) guinea pigs are examined, it it seen that the *B-* red animals are of a deeper color than the *bb* reds and that this difference is due as much to the shade of the hair as it is to the color of the skin of the ears, nose, and other exposed parts. The differences in the color of the skin may be explained on the basis of the presence of black pigment in the skin of the *B-* red animals and chocolate pigment in the skin of *bb* red individuals. It was thought that the different shades of the red hairs mights be due to the presence of black and chocolate granules in *B-* and *bb* hairs. Since in both types of hair, red granules only are present, some other explanation must be found for the difference in shade between the two types of red hair. It has already been mentioned that there is some variation in the shade of the red granules. They are somewhat lighter in *bb* red hairs than they are in *B-* red hairs. The difference was seen only in material from adult animals. This leads to the conclusion that the factors *B* and *b* may have the same kind of effect on red granules as they do on dark granules. They may produce "light" and "dark" red granules in *ee* hairs just as they produce chocolate and black granules in *E-* hairs.

CONCLUSIONS

The earliest stages of pigment formation in the hair and skin of the

guinea pig were seen in fetuses of 43 days. At this stage no difference was apparent among black, chocolate, and red pigment granules.

In older fetuses (44 to 57 days) and in adult animals, black, chocolate, and red granules were present in both the medulla and cortex of black, chocolate, and red hairs, respectively. Colorless granules, which may or may not have been homologous to the true pigment granules, were seen in white hairs. In every case, the color of the hair corresponded to the color of the granules which it contained.

Diffuse reddish pigment was seen only in the cortex of the fully formed hair in association with black, chocolate, or red granular pigment. Hence, it was present in *C*- red and dark hairs and in c^r dark hairs, but it was absent in c^r and c^a white hairs.

It is possible to explain the actions of the color determining factors in terms of their relation to granular pigment alone. The diffuse pigment may be considered as the product of the granular pigment. The following outline presents a possible explanation of the action on the hair of the color-determining factors which were studied: The factor *E* may determine the presence of dark granules. The factors *B* and *b* determine whether these granules are black or chocolate. The factor *e* in the homozygous condition may determine the presence of red granules. The factors *B* and *b* may produce "dark" and "light" red granules. In the presence of the factor *C*, both *E* and *e* may act as described above. In the presence of the factor c^r, in the homozygous condition, *E* may act as described, but *e* may produce only colorless granules, or none at all. Where the factor c^a is present in the homozygous condition, *E* does not produce dark granules and *e* does not produce red granules. Unless other factors are present, the hairs of c^ree and c^a animals are white. The action of the factors in the light and dark areas of e^p (spotted) animals corresponds to that in *e* and *E* animals, respectively. In the skin, the factors *E* and *e*, *C*, c^r and c^a may affect the amount rather than the kind of pigment present. Where there is a small amount, it is found chiefly in the extremities. The factors *B* and *b* may control the color of the pigment in the skin, and act in the presence of either *E* or *e*.

Observations on the histological and cytological origin of pigment in the guinea pig indicated that the pigment cells were ectodermal in origin and that pigment granules were not formed directly from the nucleus of the cell.

LITERATURE CITED

BOGART, R., and HEMAN L. IBSEN, 1937 The relation of hair and skin pigmentation to colour inheritance in cattle, with some notes on guinea pig hair pigmentation. J. Genet. **35**(1): 31–59.

CASTLE, W. E., and S. WRIGHT, 1916 Studies of inheritance in guinea pigs and rats. Part II. An intensive study of the inheritance of color and of other coat characteristics in guinea pigs, with especial reference to graded variations. By Sewall Wright. Carnegie Inst. Wash. Publ. No. **241**. 192 p.

EDWARDS, E. A., and S. Q. DUNTLEY, 1939 The pigments and color of living human skin. Amer. J. Anat. **65**(1): 1–33.

ESSKUCHEN, E., 1927 Über die Entstehung der Färbung der Haussäugetiere. Züchtungsk. **2**(7): 337–351.

1930 The development of pigment in cattle embryos [in German]. Z. Zücht., Reihe B, Tierzücht. u. Züchtungsbiol. **19**(2): 268–295. (Original not seen. Abstract, Expt. Sta. Rec. Wash. **65**(6): 524. Oct. 1931.)

GORTNER, R. A., 1911 On melanin. Biochem. Bull. **1**(2): 207–215.

GREMMEL, F., 1939 Coat colors in horses. J. Hered. **30**(10): 437–445.

HAWKINS, L. E., 1931 Studies on inheritance in pigeons. X. Relation of chocolate to black and dominant red. Genetics **16**: 547–573.

HUNT, H. R., and S. WRIGHT, 1918 Pigmentation in guinea pig hair. J. Hered. **9**(4): 178–181.

MAXIMOW, A. A., and W. BLOOM, 1934 A textbook of histology. 2nd ed. 662 pp. Philadelphia: Saunders.

WRIGHT, S., 1917 Color inheritance in mammals. J. Hered. **8**(5): 224–235.

1925 The factors of the albino series of guinea pigs and their effects on black and yellow pigmentation. Genetics **10**: 223–260.

1927 The effects in combination of the major color factors of the guinea pig. Genetics **12**: 530–569.

INCOMPLETE SEED FAILURE AS A RESULT OF SOMATOPLASTIC STERILITY[1]

R. A. BRINK AND D. C. COOPER
University of Wisconsin, Madison, Wisconsin

Received March 21, 1941

INTRODUCTION

NICOTIANA RUSTICA ($n=24$), used as the pistillate parent in crosses with *N. tabacum* ($n=24$), yields small, shrunken seeds, only a few of which are germinable. CHRISTOFF (1928) estimates the viability at less than one percent. We have found three germinating seeds in about 1850 tested. Two F_1 hybrids reared by CHRISTOFF were vigorous, intermediate between the parents in habit of growth, and slightly fertile. The cross, therefore, occasionally results in offspring and differs in this respect from the mating *N. rustica* ♀ × *N. glutinosa* ($n=12$) ♂ in which all the seeds die within a few days after fertilization. COOPER and BRINK (1940) have recently described the histological changes leading to seed failure in the latter case, contrasting the behavior with that in *N. rustica* which develops a full complement of seed on self-pollination. Presented here are the results of a parallel study of the *N. rustica* ♀ × *N. tabacum* ♂ mating made with a view to discovering the basis of the partial fertility.

METHODS

The procedure followed is the same as that described in the *N. rustica* × *N. glutinosa* study (COOPER and BRINK 1940). Castrated flowers of *N. rustica* were pollinated by *N. tabacum* in the greenhouse, the pistils being collected at varying intervals thereafter for histological examination of the developing seeds. Parallel matings of *N. rustica* selfed and *N. rustica* × *N. glutinosa* furnished control material. Attention was directed mainly to three points which the earlier investigations had shown to be involved in seed failure: (a) endosperm growth, (b) differentiation of conducting tissue within the seed, and (c) behavior of the nucellus. Emphasis was placed on early post-fertilization growth in view of the previous finding that the structural changes eventually leading to seed collapse originate at this time.

EXPERIMENTAL RESULTS

Early development of normal and hybrid seeds

The ovule of *N. rustica* selfed enlarges rapidly following fertilization. Measurement of two sets of five ovules each shows that the increase in

[1] Paper from the Department of Genetics, UNIVERSITY OF WISCONSIN, No. 278. The investigation was supported in part by a grant from THE ROCKEFELLER FOUNDATION. Aid was received also from WPA project in the Natural Sciences No. 8649. The authors desire to express their appreciation to PERRY BUCKNER for assistance in the histological work.

total volume between the mature embryo sac stage and the 4-celled proembryo stage is about six and one-half fold. Most of the growth is in the integument, which is the largest tissue in the young seed, and involves increase in both cell number and cell size. Corresponding increases in ovule volume occur after fertilization of *N. rustica* with *N. tabacum* and *N. glutinosa*. In fact, both classes of hybrid seeds at the 4-celled proembryo stage were found to be somewhat larger than those of *N. rustica* selfed. Too few seeds were available for measurement, however, to determine whether the differences are real. All that may be said at present is that the increase in ovule size following fertilization is at least as great in the interspecific crosses as after selfing. If not fertilized, the ovule does not expand beyond the size attained at the mature embryo sac stage.

The endosperm nucleus in normally self-pollinated *N. rustica* divides shortly after fertilization, a cell plate is formed, and the tissue becomes two-celled. Further divisions proceed in quick succession. Growth of the embryo, on the other hand, is much slower. Usually the zygote does not divide until the endosperm contains 16 to 24 cells; 2-celled proembryos are accompanied by endosperms consisting of 24 to 64 cells. The integument is enlarging noticeably at this time. It is at about the 2-celled proembryo stage that the integumentary cells lying between the distal end of the single vascular bundle within the seed and the chalazal pocket, adjacent to the endosperm, become differentiated into conducting cells. The endosperm thus becomes linked directly with the vascular system. The nucellus increases in size as the endosperm expands, but remains one-cell layer in thickness. These relatively simple morphogenetic changes foreshadow the future course of development of the seed. It is significant that they are effected early—in *N. rustica*, before the true embryo stage is reached.

Cooper and Brink (1940) have described the aberrations which occur if the sperms uniting with the *N. rustica* egg and the polar fusion nucleus are derived from *N. glutinosa* rather than from the same species. A decreased rate of endosperm growth is immediately apparent, the nucellus becomes hyperplastic, and the integumentary cells between the apex of the vascular bundle and the chalazal pocket fail to differentiate into conducting elements. Most of the seeds resulting from this wide cross cease growth within six days after pollination. The remainder collapse shortly thereafter.

The hybrid *N. rustica*×*N. tabacum* seeds are found to be similar in their early development to those from the *N. rustica*×*N. glutinosa* cross. The difference is one of degree. Compared with normal *N. rustica*, the rate of endosperm growth is lower, but the reduction is less marked than in the *N. glutinosa* cross. The endosperm is cellular from the beginning, and mitosis is regular. As in the *N. glutinosa* cross, marked hyperplasia of the nucel-

lus occurs, and a direct vascular connection to the chalazal pocket is not established.

Even at very early stages of seed development, it is found that the *N. rustica*×*N. tabacum* hybrid departs less from the normal course of development than does the *N. rustica*×*N. glutinosa* combination. This is shown by the data in table 1 relating to the 2-celled and 4-celled proembryo stages. Outline drawings were made with the aid of a camera lucida of the endosperm and the nucellus in serial sections of seeds resulting from the three different matings. The areas occupied by the respective tissues were then measured with a planimeter, and the volumes computed. Each of the values given is a mean based on seven seeds.

Several noteworthy facts are evident from the data in table 1. At the 2-celled proembryo stage the volume of the endosperm in the *N. rustica* ×*N. tabacum* hybrid is about one-third less than in *N. rustica* selfed, although it is still more than 30 percent greater than in *N. rustica*×*N. glutinosa* seeds. Likewise, the endosperm volume increases greatly in *N. rustica* selfed in passing from the 2- to the 4-celled proembryo stage and only slightly in both species hybrids. Striking also are the differences in volume of nucellus. At both respective stages the volume is least for *N. rustica* selfed and greatest for *N. rustica*×*N. glutinosa*, whereas the value for the partially fertile *N. rustica*×*N. tabacum* hybrid is intermediate.

TABLE 1

Volume (cu. mm.) of endosperm and nucellus in seeds of N. rustica selfed, N. rustica×*N. tabacum, and N. rustica*×*N. glutinosa at the 2- and 4-celled proembryo stages, respectively.*

MATING	SEED DEVELOPMENT	2-CELLED PROEMBRYO			4-CELLED PROEMBRYO		
		ENDOSPERM	NUCELLUS	E/N	ENDOSPERM	NUCELLUS	E/N
N. rustica selfed	Normal	3.037	2.062	1.47	5.135	2.818	1.82
N. rustica×*N. tabacum*	Mostly fail, a few germinable	1.979	2.745	.72	2.112	2.966	.71
N. rustica×*N. glutinosa*	All fail early	1.456	2.861	.51	1.494	3.155	.48

The balance between endosperm and nucellus, however, is the most significant relationship brought to light in table 1. This is expressed as a ratio (E/N) of the volumes of endosperm and nucellus. The E/N value for the control, *N. rustica* selfed, at the 2-celled proembryo stage is 1.47, increasing to 1.82 at the 4-celled proembryo stage. COOPER and BRINK (1940) have shown earlier that at the approximately 20-celled true embryo stage E/N is about 3.5. The ratio is much lower in both classes of hybrid seeds, being about .7 for the *N. rustica*×*N. tabacum* cross and approximately .5 for the

N. rustica×*N. glutinosa* combination. Furthermore, E/N does not increase as the hybrid seeds progress from the 2- to the 4-celled proembryo stage.

The relations discussed above are expressed in diagrammatic form in figure 1.

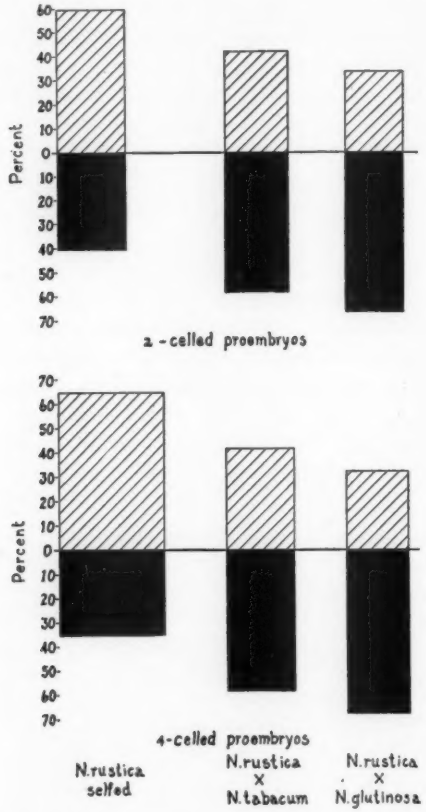
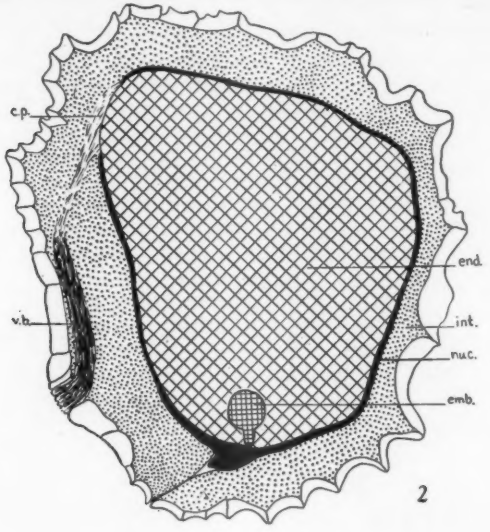


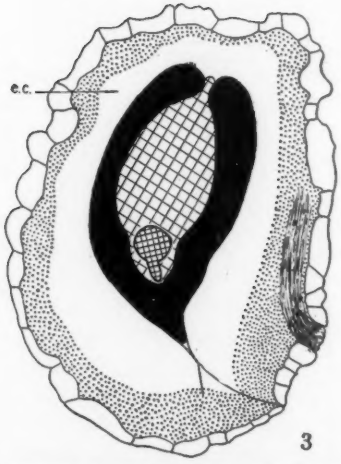
FIGURE 1.—Relative volumes of endosperm and nucellus at the 2- and 4-celled proembryo stages in *N. rustica* selfed, *N. rustica*×*N. tabacum*, and *N. rustica*×*N. glutinosa* seeds. The areas of the rectangles are proportional to volume of endosperm plus nucellus. The cross-hatched portion of the rectangle, above the zero line, corresponds to endosperm, and the black area below, to the nucellus.

These differences in the course of development are emphasized at later intervals, as is shown by an examination of material collected at 144 hours after pollination. The embryo is spherical and consists of 70 to 75 cells when *N. rustica* is selfed. A direct connection is maintained between the rapidly expanding endosperm and the vascular element of the seed (fig. 2). The

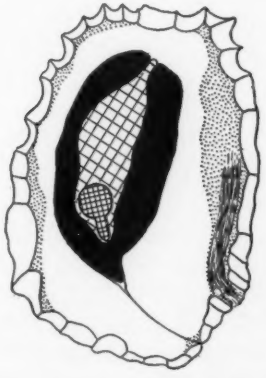
nucellus remains a single layer of cells in thickness. The cells of the integument are turgid and show no signs of disintegration.



2



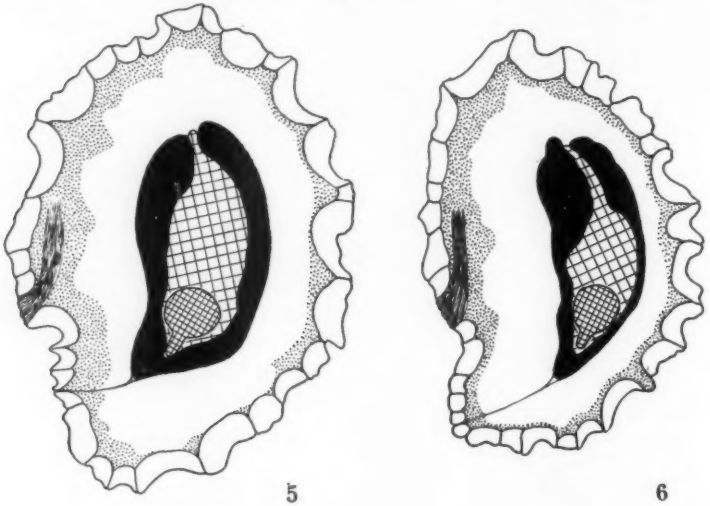
3



4

FIGURES 2 to 4.—Longitudinal sections through developing seeds 144 hours after pollination, showing the relationship of the various structures. (*int.*) integument, (*nuc.*) nucellus, (*end.*) endosperm, (*emb.*) embryo, (*v.b.*) vascular bundle, (*c.p.*) chalazal pocket.—Figure 2. *N. rustica* selfed showing the direct connection between the endosperm and the vascular bundle.—Figure 3. *N. rustica*×*N. tabacum* showing the hyperplastic nucellus.—Figure 4. *N. rustica*×*N. glutinosa*, same. The unshaded portions of the integument are composed of empty cells (e.c.). ×82.

The young seed does not develop to the same extent following the cross *N. rustica*×*N. tabacum*. The embryo is somewhat smaller and consists of 65–70 cells. The endosperm develops slowly, its volume being only a fraction of that found in the selfed series (fig. 3). The nucellus, on the other hand, is hyperplastic and consists of several layers of cells at this interval. In many instances the nucellus does not completely occlude the endosperm. The conducting tissue, however, does not establish a direct connection with the endosperm. The endosperm apparently grows at the expense of the



5 6

FIGURES 5 and 6.—Longitudinal sections through developing seeds following the cross *N. rustica*×*N. tabacum* at 288 hours, showing hyperplastic nucelli and areas of the integuments which are composed of empty cells (semi-diagrammatic). ×82.

integument. Depletion of the integument by the endosperm begins in the neighborhood of the chalazal pocket and eventually extends around the entire nucellus, as seen in figures 5 and 6.

The evidence is clear that some nutrients are moved in during development in spite of the starved appearance of the hybrid seeds. Little or no starch and fat are observable in the mature ovule, but numerous starch granules appear in the integument and nucellus shortly after fertilization. Later, the nucellus may accumulate a considerable store of food. Moreover, as impoverishment of the integument progresses, the cells on the same side of the seed as the vascular bundle retain their contents the longest. In the most extreme cases of depletion observed, only the cells in the region of the apex of the bundle are intact. It would appear, therefore, that while foods

continue to move into the hybrid seeds, the rate of translocation is much too low to balance the losses of the integument to the hyperplastic nucellus and the enclosed endosperm and embryo.

Only a very few actively developing seeds are present at this interval when *N. glutinosa* is used as the staminate parent. Even though such seeds are appreciably smaller than those in the *N. rustica*×*N. tabacum* cross, the embryos are of a similar size. The endosperm, on the other hand, is very poorly developed even in those instances where it has not become completely separated from the integument by the proliferating nucellus (fig. 4). Practically all the cells of the integument with the exception of those immediately adjacent to the vascular bundle are completely empty.

Seeds of varying sizes are found at 288 hours after pollination in the cross *N. rustica*×*N. tabacum*. These range from completely aborted remnants to those with turgid endosperms and growing embryos. Even among those which are continuing to develop there is a wide variation in size (fig. 5, 6). There is likewise a considerable range in the amount of endosperm present. The cells of the integument, with the exception of those at the periphery and those in the region of the vascular bundle, are empty and more or less collapsed.

Delayed collapse of the N. rustica×*N. tabacum seeds*

It is evident from the foregoing account that the course of development entered upon by the *N. rustica*×*N. tabacum* seed is similar to that which leads to early collapse in the case of *N. rustica*×*N. glutinosa*. The essential difference between the two hybrids is in time of seed collapse. The *N. rustica*×*N. glutinosa* seeds all abort within about ten days after fertilization. Many of the *N. tabacum* hybrid seeds cease growth during this period also, but others continue development for varying periods up to the time the capsule is ripe.

The immediate cause of death of the *N. rustica*×*N. glutinosa* seeds was attributed by COOPER and BRINK (1940) to starvation of the endosperm through interruption of the food supply by overgrowth of the nucellus. It was pointed out that the histological evidence indicates that in addition to consuming nutrients which would normally pass to the endosperm and embryo, the hyperplastic nucellus also tends to block the inward movement of these materials. Frequently, although not invariably, the endosperm of collapsing seeds is found to be completely occluded by the overgrown nucellus. That is, the gap adjacent to the chalazal pocket through which the nutrients normally appear to flow to the endosperm is closed by proliferation of the neighboring nucellar cells. This condition is the climax of the structural changes associated with somatoplastic sterility in tobacco.

Even though collapse of the seed often intervenes before the nucellar gap

closes entirely, the frequency of occluded endosperms may be used as a measure of the intensity of the developmental changes leading to somatoplastic sterility in this plant. Counts were made, therefore, on seeds of the two respective hybrids at the 8- to 16-celled embryo stage to determine the proportions in which the nucellar gap is closed. Collapse is rather frequent in both hybrids at this time, but the counts were based on seeds which had not yet shown clear signs of breakdown.

As shown in table 2, the nucellar gap was still open in 75 percent of the *N. rustica*×*N. tabacum* seeds at this stage as compared with only 26 percent of the cases in the *N. rustica*×*N. glutinosa* cross. The gap is open in all the selfed *N. rustica* seeds, there being no hyperplasia of the nucellus associated with this fully fertile mating. According to this criterion, therefore, the grade of somatoplastic sterility is definitely lower in the *N. tabacum* hybrid than the *N. glutinosa* cross. The data on occlusion are also in accord with expectation based on the E/N relations in the young fertile ovules, as discussed above. All these data point to the conclusion that the developmental changes associated with somatoplastic sterility progress more slowly in the *N. rustica*×*N. tabacum* seeds than in the seed from the corresponding *N. glutinosa* cross.

TABLE 2

Distribution with reference to open and closed nucellar gaps of N. rustica selfed, N. rustica×*N. tabacum and N. rustica*×*N. glutinosa seeds at the 8- to 16-celled embryo stage.*

MATING	NUCELLAR GAP		
	NUMBER OPEN	NUMBER CLOSED	PERCENT OPEN
N. rustica selfed	all	0	100
N. rustica×*N. tabacum*	68	22	75
N. rustica×*N. glutinosa*	27	76	26

Development of viable seeds in the N. rustica×*N. tabacum cross*

COOPER and BRINK (1940) observed that exceptional *N. rustica*×*N. glutinosa* seeds, living as long as nine days after fertilization, showed a narrow, finger-like portion of the endosperm projecting through the nucellar gap into the chalazal pocket. They suggested that this might be the basis for the increased duration of growth following other matings in which collapse of the seed characteristically occurs at later stages of development. The present investigation affords corroborative evidence that this anatomical relationship is an important factor affecting survival. It is probable that the few shrunken but germinable seeds which are found at maturity after the *N. rustica*×*N. tabacum* cross owe their continued development to this adventitious connection of the endosperm with the integument.

Numerous still viable *N. rustica*×*N. tabacum* seeds were examined at intervals varying from six to 15 days beyond fertilization. The most advanced of these seeds had reached approximately the mid-period of development. The embryos in some of them showed the beginnings of the cotyledonary outgrowths. Since the hardened testa interferes with sectioning of still older seeds, later stages were not examined.

Without exception, the endosperm in these longer persisting seeds still maintains a connection with the chalazal pocket through the nucellar gap. The connecting strand of endosperm tissue is of small circumference and is sometimes of considerable length, as shown in figure 6. There is no development of the vascular tissue beyond that found in the mature ovule. The growth of the hybrid endosperm and embryo is at the expense of the integument. The cells of the latter tissue adjacent to the chalazal pocket lose their contents rather early, and as the seed becomes older, a large part of the integument becomes depleted. The restricted growth and impoverishment of this outer tissue is reflected in the excessively shrunken condition of the mature seed.

Thus there is a basic difference in the mechanism by which the nutrients within normal *N. rustica* and *N. rustica*×*N. tabacum* seeds are distributed. In the former, differentiation into conducting tissue of the integumentary cells between the apex of the vascular bundle and the chalazal pocket enables a portion of the nutrients moving into the seed to pass directly to the endosperm. The latter nourishes the embryo, which it encloses. Presumably, nutrients also diffuse from the conducting cells into the integument, supporting growth in this tissue and in the nucellus. In the hybrid seeds, on the other hand, the differentiation does not occur, so that the terminus of the vascular strand remains in the integument. The integument, consequently, is the recipient of all the incoming food materials. A secondary mechanism for nourishing the embryo, however, may come into play. The endosperm may project through the nucellar gap, making contact with the seed coat at that point, and thus obtain food material from the integument sufficient for continued growth. The integumentary cells are unable to replace fully the materials given up to the endosperm, however, and an increasing number of them become deprived of their contents as development proceeds. Most of the hybrid seeds fail under these conditions, but a few may form functional embryos. An essential anatomical difference between the normal seed and the hybrid, therefore, is that in the former the endosperm is in a terminal position with reference to the vascular element, whereas in the cross, undifferentiated integumentary tissue is interposed between the endosperm and the end of the vessels. The normal and secondary mechanisms by which the nutrients are partitioned in the seed are illustrated in figure 7.

DISCUSSION

The evidence presented above shows that the *N. rustica*×*N. tabacum* cross represents a case of incomplete somatoplastic sterility. The post-fertilization development of the ovule departs from the normal course in the same way as in the completely abortive *N. rustica*×*N. glutinosa* seeds,

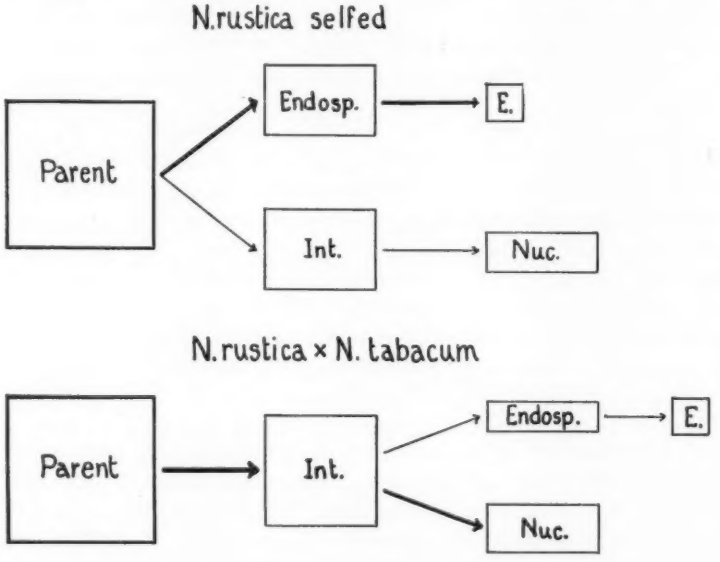


FIGURE 7.—Flow chart illustrating the presumed distribution of nutrients within normal *N. rustica* seeds (above) and *N. rustica*×*N. tabacum* hybrid seeds (below). Whereas all the nutrients entering the hybrid seeds are discharged into the integument (Int.), a portion passes directly to the endosperm (Endosp.) in normal seeds. The respective relations of embryo (E.) and nucellus (Nuc.) to these tissues are shown.

but in a lesser degree, so that a few of the seeds may attain a germinable condition. COOPER and BRINK (1940) found that the *N. rustica*×*N. glutinosa* hybrid seeds differ profoundly in their histology from those of normal *N. rustica* and that the changes are of a sort which would be expected to alter the partition of nutrients between the endosperm and the surrounding tissues. The behavior of the *N. rustica*×*N. tabacum* hybrid demonstrates how a fertile ovule thus modified in its physiological anatomy may develop into a germinable seed. Disorganization of the primary mechanism for nourishing the embryo does not necessarily result in sterility; the residual structure may still be capable of becoming a functional seed.

A primary requirement for continued growth of the hybrid seeds is that

the endosperm retain a direct association with the integument through the nucellar gap. If the nucellar gap remains open, sufficient nutrients may move in from this tissue to maintain the endosperm as a functioning organ. The integument, however, becomes exhausted in the process.

As compared with the *N. rustica*×*N. glutinosa* hybrid, in which all the seeds fail, the *N. rustica*×*N. tabacum* cross shows a more rapid early growth of the endosperm and, at later stages, a higher proportion of seeds in which the endosperm maintains contact with the integument through the chalazal gap. The more rapid early development of the endosperm in the *N. tabacum* hybrid is probably the basic factor in the higher E/N ratio observed and in maintaining a direct connection with the integument in the few seeds which persist. Early post-fertilization behavior appears to be doubly critical for seed survival in the sense that if the primary morphogenetic changes normally occurring at this stage fail, the essential character of the secondary mechanism for nourishing the embryo is concurrently determined.

The new facts further emphasize the responsiveness of the endosperm to variations in genotypic constitution and the basic significance of the tissue in early seed development. Not only does the endosperm nourish the enclosed embryo, but it is now apparent that it also has an important morphogenetic function. The establishment of conducting tissue between the vascular strand and the chalazal pocket is dependent upon it in the tobacco ovule. The success or failure of this highly localized early post-fertilization event profoundly influences subsequent development because of the effect on distribution of nutrients within the seed.

The rapid enlargement of the ovule following syngamy suggests that growth promoting substances are formed within the embryo sac which diffuse into the surrounding tissues. It is probable that these substances are produced by the endosperm rather than the embryo because of the much more rapid growth of the former. Differentiation of the integumentary cells between the apex of the vascular bundle and the chalazal pocket into conducting cells is one of the primary responses of the ovule to these endosperm secretions. COOPER and BRINK (1940) point out that it is along this path that the maximum concentration of nutrients passing to the fertile embryo sac probably occurs. The extension of the vascular tissue which ensues may be considered as a reaction of these well nourished cells to the substances diffusing from the endosperm. Whether distant outcrossing alters the endosperm secretion qualitatively or only reduces its amount below the effective threshold cannot be stated. The rate of growth of the hybrid endosperm is lower than that of the normal, so that the amount of the secretion is probably reduced whether or not is it also changed in kind.

Mitosis is regular in the endosperm of these Nicotiana species hybrids.

The young endosperm in some plants is particularly prone to collapse on fixation and thus yield an unrepresentative histological picture (cf. BOYES and THOMPSON 1937); and it seems probable that the gross abnormalities in nuclear and cell division which are occasionally reported originate in this way. Examination in our laboratory of numerous preparations of endosperm tissue of both monocotyledons and dicotyledons leads us to the view that any interpretation of seed failure which assumes that mitotic irregularities are responsible is clearly open to question.

Most of the studies which have been made of partial and complete sterility resulting from seed collapse have dealt with the arrest of growth at stages well beyond fertilization. Doubtless much early abortion has been overlooked because of the technical difficulty of separating it from nonfertilization. Nevertheless, it is clear that failure is not limited to one period but may occur at any time in the growth of the seed. COOPER and BRINK (1940) have pointed out the parallel between their findings on early seed failure in the *N. rustica*×*N. glutinosa* cross and the observations of RENNER (1915) on Oenothera, BRADURY (1929) on the sour cherry, *Prunus cerasus*, and SATINA and BLAKESLEE (1935) on the hybrid between *Datura stramonium* and *D. metel*. All these cases exhibit one or more of the essential early post-fertilization features of the phenomenon we have termed somatoplastic sterility. The question now to be considered concerns the extent to which deferred seed failure also falls in the same general category.

The evidence on the *N. rustica*×*N. tabacum* hybrid is significant in this connection is establishing the fact that the early post-fertilization morphogenetic changes characteristic of somatoplastic sterility may not prove to be lethal immediately. The collapse which occurs later, however, may be traced directly to the changes manifested in the young fertile ovule. In other words, the mechanism of seed failure is the same whether collapse occurs early or late. The life span of the growing seed is determined by the speed with which the abnormal type of development proceeds. This finding creates a presumption in favor of the view that arrested growth in relatively advanced seeds of other species is frequently due to a set of causes similar to those described.

This question is of considerable interest in several of the orchard plants in which seed failure has received much attention because of the relationship between it and development of the associated fruit. Analysis of the reproductive problem in these cultivated forms has proved difficult because of the diversity of more or less interrelated phenomena involved. Variations affecting fertility and fruitfulness occur at all stages in the reproductive cycle from differentiation of the flower bud onward; and behavior at one phase may condition the reaction of the plant at succeeding phases. Furthermore, there is a correlation between one reproductive cycle and the

next in varieties prone to fruit in alternate years. Nevertheless, considerable progress has been made in resolving the complex into its elements; and it is proposed now to inquire to what extent the observed variations in seed development may be interpreted in terms of somatoplastic sterility.

WAITE (1895), in an early investigation on pollination in the pear, reported that fruits resulting from selfing not only contain few seeds but that these are usually abortive. Cross-fertilization, on the other hand, gives rise to fruits well supplied with sound seeds. Other workers have since observed this same relation in the apple and the pear. Since many varieties in these species are partially self-incompatible, there are probably two distinct factors responsible for the reduced seed formation, as COOPER and BRINK (1940) find in alfalfa. The frequency of ovules becoming fertile in the latter plant is lowered as a result of restricted pollen tube growth, and secondly, many fertile ovules fail to develop into mature seeds because of somatoplastic sterility. The assumption that somatoplastic sterility resulting from a weakened condition of the endosperm as a result of inbreeding is responsible for the shrunken seeds in self-pollinated pome fruits is not wholly gratuitous. BRYANT (1935) has shown, for example, that development of the endosperm in the apple is definitely retarded after selfing and that, following compatible out-crossing, the collapse of seeds tends to occur later and is reduced in frequency. It may be anticipated that the advantage now generally recognized in making provision for cross-pollination in commercial plantings of several of the orchard fruits will be found to rest not only upon the higher incidence of fertilization that results but also upon the lowered rate of failure of seeds containing hybrid rather than inbred endosperms.

A relatively small proportion of the flowers on a normally blossoming fruit tree give rise to mature fruits. HEINICKE (1917) states that it is usually less than ten percent in the apple. The remainder are shed, often in three rather irregular waves termed first, second, and third or June "drops," beginning shortly after blossoming starts and terminating several weeks later. The causes of this drastic reduction in fertility have received considerable study. The main emphasis has been placed upon the nutritional relations, which HEINICKE (1917), ROBERTS (1926), and others have shown to be important. Our present attention is directed to a limited phase of the question—namely, the nature of the seed failure which is associated with abscission of the fruit.

MÜLLER-THURGAU (1898) showed that the June drop fruits usually contain embryos which have ceased to grow. The arrested growth of embryos was emphasized by KRAUS (1915) as a major factor in reducing fertility in this class of plants. DORSEY (1919), working with the plum, found that the flowers falling in the first drop possessed aborted pistils, the frequency

of which, like fruit bud formation, tended to be correlated with the size of the preceding crop of fruit. The second drop, occurring from two to four weeks after blooming, involved the remaining pistils in which fertilization had not taken place. The third drop is characterized by fruits of larger size in which fertilization has occurred but embryo development has stopped. ROBERTS (1926) also observed that in the apple the first drop involves infertile flowers. Practically all second drop fruits, however, showed evidence of fertilization. The more advanced drop fruits contained seeds in which the embryo was partially developed. Additional evidence is presented by DETJEN (1926) showing that in the apple, plum, peach, nectarine, and sour cherry, the fruits falling somewhat beyond the blossoming stage contain fertile ovules. MURNEEK (1937) has recognized four waves of drops in the apple in Missouri. Embryos are frequently present in the small second drop fruits but are retarded in growth. The numerically smaller third and fourth drops are made up of apples $\frac{1}{2}''$–$1''$ in diameter containing partially developed seeds.

BRADBURY (1929) examined 250 third drop fruits of the sour cherry and found embryos in all but two of them. This investigator's histological studies provide the clearest evidence at present available that the seed failure associated with premature dropping of the fruit in orchard plants is of the same type as that described herein for the *N. rustica*×*N. tabacum* cross. The fact of primary importance which BRADBURY presents in this connection is that the seeds in dropped fruits have a weakly developed endosperm and an overgrown nucellus.

DORSEY (1919) observed that the number of fruits reaching maturity in the Compass variety of plum differed greatly, depending upon whether it was pollinated with the Yellow Egg or the Burbank variety. Of 1327 Compass flowers pollinated by Yellow Egg, 652 fruits began development, but only eight matured. When Burbank was used as the pollen parent on the same pistillate variety, of 175 flowers pollinated, 116 set fruit and 114 matured. DORSEY reports further, "A characteristic of all the seeds dissected in the cross Compass×Yellow Egg was the small amount of endosperm present."

There are definite indications in the work of DORSEY (1919), BRADBURY (1929), and BRYANT (1935), therefore, that somatoplastic sterility is a frequent cause of seed collapse in the orchard fruits. Confirmation of this view and a detailed knowledge of the manner in which the phenomenon is manifested in these rosaceous plants awaits further investigation.

Assuming that somatoplastic sterility is the operating mechanism, one of the important questions to be elucidated in the orchard fruits is the manner in which variations in the plane of nutrition affect the incidence of seed failure. It may be pointed out at this time that fluctuations in the amount

of seed failure according to the nutrient level are consistent with the somatoplastic sterility hypothesis. The genetic composition of the endosperms formed in these highly heterozygous plants may be expected to differ widely. By virtue of these inherent differences, some are more able than others on a given plane of nutrition to support normal seed development. As the nutrient level of the seed declines, due to a heavy set of fruit, unfavorable position on the plant, and the like, it may be expected that an increasing proportion of the endosperms will fall into the ineffective class. DORSEY (1919) has expressed a similar idea, in terms of the embryo, however, rather than the endosperm.

TUKEY (1933a, 1933b) and TUKEY and LEE (1937) in a significant series of studies, find that the early ripening varieties of the sweet cherry (*Prunus avium*), sour cherry, and peach (*P. persica*) are characterized by defective or abortive seeds. Varieties which mature at successively later times form larger, often viable, seeds whose embryos are correspondingly plumper and firmer. Three developmental stages between flowering and maturity of the fruit are recognized which TUKEY and LEE (1937) characterize in the following way for the peach. In stage I, extending for about 50 days following full bloom in all varieties, rapid growth of the fruit occurs, whereas the embryo remains microscopic in size. During state II, development of the fruit is retarded and the embryo begins a cycle of growth which continues until it attains the maximum size for the variety. The duration of stage II varies directly with the lateness of maturity of the variety (five days for Greensboro, an early sort, and 42 days for Chili, a late variety). The final swell of the fruit takes place in stage III. The onset of stage III, varying in time according to the length of stage II, finds the embryos in early varieties still small, whereas those of late varieties are nearly full size. During stage III there is an accumulation of reserve foods, particularly fats, in the embryo. The amount stored is small in the case of early varieties with abortive seeds and markedly higher in late varieties forming viable seeds.

The extensive body of evidence which TUKEY and his associates have obtained is especially significant for an understanding of the causal relationships between seed and fruit formation. Since our investigations are concerned primarily with the seed, we shall limit comment mainly to this structure. If TUKEY's work falls short of pointing the way to a satisfactory explanation of the sterility found, this is due, in our judgment, to the relative paucity of facts regarding stage I. The data regarding differentiation and growth of the young seed in orchard fruits are fragmentary, as we pointed out earlier. The limited evidence available, however, indicates that early post-fertilization behavior in these plants is subject to the same disturbances we have described for Medicago (BRINK and COOPER, 1940) and as are now reported for the incompletely sterile *N. rustica*×*N. tabacum*

cross. One may expect, therefore, that the course of development leading to abortive or defective seeds in the drupes has its inception also in an unbalance in early growth between the endosperm and adjacent maternal tissues. The unbalance, being manifest during a period when morphogenetic changes affecting the distribution of nutrients are occurring determines the subsequent behavior of the seed. The formative changes in the seed governed by the endosperm take place during stage I; presumably, a stage II of normal duration is contingent upon their completion during this previous period. If development of the seed in stage I is not regular, then various secondary effects may ensue, one of which, in the drupes, appears to be a hastening of maturity of the fruit. It is not to be inferred, however, that earliness of maturity in this material is governed solely by the behavior of the seed. The influence of the maternal genotype, as such, on the seed and fruit is also to be considered. This brings us to our final point.

Implicit in our interpretation of seed collapse is the assumption that somatoplastic sterility is influenced by the maternal genotype as well as by that of the endosperm. Normal seed development is considered to be contingent upon a certain balance between the reaction norms of these genotypically diverse tissues. Two-thirds of the inheritance carried by the endosperm is a reduplicated sample half of the maternal genotype. This means that the genetic make-up of the endosperm is quantitatively much more like that of the associated maternal tissue than if the inheritance were received equally from the two parents, as in the embryo. Even with the "adaptive" possibilities inherent in this mechanism, however, it is probable that the mean difference in the reaction norms of maternal tissues of the seed and endosperm varies significantly from one variety to another. For the sake of simplicity, one may think of the maternal genotype in this connection as tending to promote hyperplasia of the nucellus and integument during early seed development and the various possible associated endosperm genotypes as opposing this process. We are suggesting that the disparity in strength of these two forces differs somewhat according to the genotype of the maternal parent. Although conclusive evidence for this view is not at hand, some facts are available that seem to validate it.

The formation of certain seedless fruits, for example, requires that fertilization occur and that the seed develop for at least a short period. The seed then collapses but the fruit continues to grow. PEARSON (1932) finds that this behavior characterizes the Sultanina (Thompson Seedless), Sultana, and Black Monukka grapes. The work of OLMO (1934), STOUT (1936), and others shows that the phenomenon is common in Vitis. Between the seedless and highly fertile varieties different grades of seedlessness are recognized, a given clone being relatively constant in this respect. OLMO (1934) emphasizes the interesting fact that the degree to which seeds develop is

governed by the maternal genotype and is independent of the source of pollen. STOUT (1936) makes the same statement.

The histology of seed collapse in the grape has not been fully worked out; but from the accounts given by PEARSON (1932) and STOUT (1936) it appears that excessive proliferation of the nucellus and integuments in association with a weakly growing endosperm may be involved. STOUT's (1936) figures 22, 23, and 46, relating to seedless clone No. 10918, suggest this explanation. STOUT observes in this case that month old seeds may contain a small, globular embryo, "a narrow central cylinder of rather few endosperm cells and much nucellar tissue." It is probably significant also that the inner integument in this clone at anthesis abnormally protrudes beyond the outer integument and undergoes much further proliferation after fertilization. This is not an isolated instance, for PEARSON (1932) previously reported the same condition in the seedless Sultanina, Sultana, and Black Monukka varieties. Is this to be construed as structural evidence of a predisposition toward abnormal growth of the maternal tissues of the seed regardless of the genetic constitution of the associated endosperm? Provisionally, the fact may be so interpreted. Further study of this aspect of somatoplastic sterility is needed, however, and the grape appears to be a plant for which the required quantitative evidence might be obtained.

SUMMARY

A histological study of seed development following the mating *Nicotiana rustica* ♀ × *N. tabacum* ♂ is reported. Most of the hybrid seeds collapse at various stages before maturity of the capsule; a small fraction, however, may attain a germinable condition. The behavior is compared with that in *N. rustica* selfed, in which seed formation is normal, and with *N. rustica* ♀ × *N. glutinosa* ♂, in which all the seeds abort at an early stage.

The course of development of the *N. rustica* × *N. tabacum* seeds is similar to that in the *N. rustica* × *N. glutinosa* mating in that (a) endosperm growth is retarded, (b) pronounced hyperplasia of the nucellus occurs, and (c) the integumentary cells lying between the apex of the vascular bundle and the chalazal pocket fail to differentiate into conducting elements. The difference between these two kinds of seeds with reference to endosperm growth and hyperplasia of the nucellus is quantitative, the *N. tabacum* hybrid showing a less abnormal development than the *N. glutinosa* hybrid.

The two kinds of hybrid seeds differ from each other and from normal *N. rustica* seeds even at the 2-celled proembryo stage in relative volumes of endosperm and nucellus (E/N ratio). *N. rustica*, selfed, shows the highest value (1.47), *N. rustica* × *N. glutinosa*, the lowest (.51), and *N. rustica* × *N. tabacum* is intermediate (.72).

At the 8- to 16-celled embryo stage the proportions of seeds in which the

endosperm is occluded by overgrowth of the nucellus were as follows: *N. rustica*, selfed, none; *N. rustica*×*N. tabacum*, 22 percent; *N. rustica*×*N. glutinosa*, 76 percent.

In all *N. rustica*×*N. tabacum* seeds continuing growth beyond the early stages the endosperm was found to be in direct association with the integument through a narrow gap in the nucellus at the chalazal end.

The endosperm and embryo in persisting *N. rustica*×*N. tabacum* seeds grow at the expense of the integument, the cells of which become depleted of their contents during the process.

The *N. rustica*×*N. tabacum* hybrid is interpreted as a case of incomplete somatoplastic sterility, the developmental changes associated with this phenomenon usually, but not invariably, proceeding to a point which results in seed failure.

The present findings are discussed in relation to seed failure in some other plants, particularly certain cultivated fruits.

LITERATURE CITED

BOYES, J. W., and W. P. THOMPSON, 1937 The development of the endosperm and embryo in reciprocal interspecific crosses in cereals. J. Genet. **34**: 203–227.

BRADBURY, DOROTHY, 1929 A comparative study of the developing and aborting fruits of *Prunus cerasus*. Amer. J. Bot. **16**: 525–542.

BRINK, R. A., and D. C. COOPER, 1940 Double fertilization and development of the seed in angiosperms. Bot. Gaz. **102**: 1–25.

BRYANT, L. R., 1935 A study of the factors affecting the development of the embryo sac and the embryo in the McIntosh apple. Univ. New Hamp. Agric Exp. Sta. Bull. **61**: 1–40.

CHRISTOFF, M., 1928 Cytological studies in the genus Nicotiana. Genetics **13**: 233–277.

COOPER, D. C., and R. A. BRINK, 1940a Partial self-incompatability and the collapse of fertile ovules as factors affecting seed formation in alfalfa. J. Agric. Res. **60**: 453–472.

1940b Somatoplastic sterility as a cause of seed failure after interspecific hybridization. Genetics **25**: 593–617.

DETJEN, L. R., 1926 Physiological dropping of fruits. I. Relation of pollination, fertilization and embryo abortion to abscission in fruits. Delaware Agric. Exp. Sta. Bull. **143**: 1–47.

DORSEY, M. J., 1919 A study of sterility in the plum. Genetics **4**: 417–488.

HEINICKE, A. J., 1917 Factors influencing the abscission of flowers and partially developed fruits of the apple (*Pyrus malus* L.). Cornell Univ. Agric. Exp. Sta. Bull. **393**: 43–114.

KRAUS, E. J., 1915 The self-sterility problem. J. Hered. **6**: 549–557.

MÜLLER-THURGAU, H., 1898 Abhängigkeit der Ausbildung der Traubenbeeren und einiger anderer Früchte von der Entwicklung der Samen. Landw. Jahrb. Schweiz. **12**: 135–205.

MURNEEK, A. E., 1937 Pollination and fruit setting. Missouri Agric. Exp. Sta. Bull. **379**: 1–28.

OLMO, H. P., 1934 Empty-seededness in varieties of *Vitis vinifera*. Proc. Amer. Soc. Hort. Sci. **32**: 376–380.

PEARSON, H. M., 1932 Parthenocarpy and seed abortion in *Vitis vinifera*. Proc. Amer. Soc. Hort. Sci. **29**: 169–175.

RENNER, O., 1915 Befruchtung und Embryobildung bei *Oenothera Lamarckiana* und einigen verwandten Arten. Flora **107**: 115–150.

ROBERTS, R. H., 1926 Apple physiology. Growth, composition, and fruiting responses in apple trees. Wisc. Agric. Exp. Sta. Res. Bull. **68**: 1–72.

SATINA, S., and A. F. BLAKESLEE, 1935 Fertilization in the incompatible cross *Datura stramonium* and *D. metel*. Bull. Torrey Bot. Cl. **62**: 301–312.

STOUT, A. B., 1936 Seedlessness in grapes. N. Y. Agric. Exp. Sta. Tech. Bull. **238**: 1–68.

TUKEY, H. B., 1933a Embryo abortion in early-ripening varieties of *Prunus avium*. Bot. Gaz. **94**: 433–468.

1933b Growth of the peach embryo in relation to growth of fruit and season of ripening. Proc. Amer. Soc. Hort. Sci. **30**: 209–218.

TUKEY, H. B., and F. A. LEE, 1937 Embryo abortion in the peach in relation to chemical composition and season of fruit ripening. Bot. Gaz. **98**: 586–597.

WAITE, N. B., 1895 The pollination of pear flowers. Bull. U. S. Dept. Agric. Div. Veg. Path. **5**: 1–110.

A RELATION BETWEEN LARVAL NUTRITION AND THE FREQUENCY OF CROSSING OVER IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER

JAMES V. NEEL

Dartmouth College, Hanover, New Hampshire

Received for publication April 1, 1941

THE frequency of meiotic crossing over in Drosophila females is known to be influenced by such non-genetic factors as the age of the fly, the temperature prevailing during larval, pupal, and adult life, exposure to X-rays, radium radiations, and ultraviolet radiation, treatments designed to render the female temporarily sterile, and the length of time spent in the larval stadium (for reviews of the literature on this subject cf. BRIDGES 1929, STERN 1933, LUDWIG 1938). The investigation described below was undertaken to determine whether or not another environmental factor affecting the frequency of genetic recombination in *D. melanogaster*—and one which could be a source of complication in the last of the above mentioned correlations—might, under certain conditions, be the availability of food during the larval stage.

This question has been briefly touched upon by several earlier investigators. PLOUGH (1917) was unable to detect any influence of prolonged partial starvation of larvae upon crossing over. GOWEN (1919) noted differences in the amount of crossing over shown by flies raised on fermented banana and genetically comparable individuals raised on an artificial food mixture consisting of starch, sugar, peptone, yeast, and water, but these differences were not certainly significant. Finally, the papers of SEREBROVSKY (1927) and BERGNER (1928) contain data which can be interpreted as indicating that food conditions have an effect on the frequency of genetic recombination, although these data, for reasons which will be discussed below, do not permit a satisfactory evaluation of the role of nutrition.

The results of this investigation were reported in a preliminary fashion at the 1940 Christmas meetings of the Genetics Society of America (NEEL 1941).

MATERIALS AND METHODS

The first step in the present work involved a mass mating of approximately 100 wild type females with an equal number of rucuca males. The latter were homozygous for a well known combination of third chromosome genes—namely, roughoid (*ru*, 0.0), hairy (*h*, 26.5), thread (*th*, 43.2), scarlet (*st*, 44.0), curled (*cu*, 50.0), stripe (*sr*, 62.0), sooty (e^s, 70.7), and claret (*ca*, 100.7). When the females were laying freely, eggs were collected

TABLE 1

A summary of the types of third chromosomes recovered from control and experimental flies during successive periods.

PERIOD	REGION(S) INVOLVED IN THE EXCHANGE																										TOTAL
	0		1		2		3		4		1, 2		1, 3		1, 4		2, 3		2, 4		2, 4		1, 2, 3		1, 2, 4		1, 3, 4		
	+	*h*	*h*		*h*		*h*		*h*		*h*		*h*		*h*		*h*		*h*										
	+	*th*		*th*	*th*		*th*		*th*			*th*		*th*		*th*	*th*		*th*				*th*		*th*			*th*	
	+	*cu*		*cu*		*cu*	*cu*		*cu*		*cu*			*cu*		*cu*		*cu*		*cu*								*cu*	
	+	*sr*		*sr*		*sr*		*sr*	*sr*		*sr*		*sr*			*sr*	*sr*				*sr*	*sr*	*sr*			*sr*			
	+	*e*^{a}		*e*^{a}		*e*^{a}		*e*^{a}		*e*^{a}	*e*^{a}		*e*^{a}		*e*^{a}		*e*^{a}		*e*^{a}				*e*^{a}	*e*^{a}				*e*^{a}	
A. CONTROL																													
1	310	277	76	91	22	14	40	53	37	36	2	4	13	16	8	14	1	3	3		1				1				1022
2	475	441	93	81	12	9	38	43	37	48	2	2	8	8	9	8	1	1	1										1317
3	381	327	66	75	6	4	28	33	33	52			5	8	10	5				2									1035
4	377	332	79	68	13	6	46	49	40	61		1	7	5	10	5	1						1						1101
5	395	300	55	75	9	7	37	32	25	32	2		5	8	9	3													994
6	286	263	63	45	5	7	16	27	27	25		1	8	6	9	8													796
7	167	128	27	32	2	1	11	13	14	28	1		6	3	2					1	1								437
8	98	74	20	18			11	9	7	7			2	2	1														249
																													6951
B. EXPERIMENTAL																													
1	432	375	101	99	24	21	70	60	52	66	4	4	15	15	14	12	1	2	3		3				1		1	1	1376
2	563	535	118	132	15	16	76	59	54	81	2	2	18	21	13	11	2	1		1	2				1				1723
3	483	447	100	123	13	18	60	52	46	63	1		10	9	8	13	1	1	1										1449
4	362	295	73	66	11	11	40	30	36	42	2	4	7	5	19	11		1	1		1								1026
5	229	223	53	41	2	3	26	23	34	29			3	7	7	4													684
6	155	149	36	42	3	4	18	17	18	20			5	5	5					1									478
7	91	77	21	25	1	1	7	8	13	12			3	2	1	3													265
8	54	34	9	9	1		13	2		10					3	1													136
																													7137

at four hour intervals and placed in lots of 60 to 70 on well yeasted corn-meal-molasses agar contained in one-fourth pint milk bottles. Development took place at 26°C. At a mean egg-larval age of 70 hours the larvae were divided into two groups. One group, the controls, completed development under the previously existing conditions. Larvae belonging to the other group were placed in vials containing nothing but Kleenex (an absorbent cleansing tissue) which had been moistened with water. A high proportion of the larvae so treated formed puparia, and puparium formation and the subsequent eclosion took place at approximately the same time or even slightly earlier in the experimental group than in the controls. This observation agrees with the findings of ALPATOV (1930) and BEADLE, TATUM, and CLANCY (1938). The mean weight of 58 newly eclosed females developing from control larvae was 1.11 mg, while that of 171 females developing from partially starved larvae was 0.64 mg.

TABLE 2

The percent of crossing over in the different regions during successive periods.

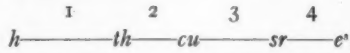
REGION	PERIOD							
	1	2	3	4	5	6	7	8
				A. CONTROL				
1	22.0	16.0	16.3	16.0	15.8	17.6	16.2	17.3
2	4.8	2.1	1.2	2.0	1.8	1.6	1.1	0.0
3	12.4	7.6	7.2	9.9	8.3	7.2	7.8	9.7
4	9.8	7.8	9.8	10.5	6.9	8.6	10.5	6.0
Total	49.0	33.5	34.5	38.4	32.8	35.0	35.6	33.0
				B. EXPERIMENTAL				
1	19.4	18.4	18.2	18.2	16.8	19.4	20.8	16.2
2	4.4	2.3	2.4	2.9	0.7	1.7	0.8	0.7
3	12.2	10.4	9.2	9.1	8.6	9.4	7.5	11.0
4	11.1	9.5	9.0	10.7	10.8	9.2	10.9	10.3
Total	47.1	40.6	38.8	40.9	36.9	39.7	40.0	38.2

Female flies developing from the two types of larvae were collected within 12 hours of eclosion and each placed for two days in a vial containing about a dozen rucuca males. The largest of the control females and the smallest of the experimental group were chosen for this backcross. The two day period in a vial with an excess of the rucuca males was found to be necessary to insure the fertilization of even a minority of the females. At the end of the two days, each female, together with the dozen or so males, was transferred to a one-fourth pint milk bottle containing well yeasted food, allowed to remain there three days, then transferred to a fresh bottle where she was allowed to remain a similar period of time, and

so on until eight successful transfers had been effected. From the offspring of this back cross the frequency of crossing over between *h*, *th*, *st*, *cu*, *sr*, and e^s during successive periods in the two types of females was determined. Only those females were used in the calculation of recombination frequencies which began to lay during the first three day period and continued to lay during at least six successive periods. Six out of 20 attempted control matings and 11 out of 43 matings involving females which had developed from partially starved larvae met this condition.

RESULTS

Although crossing over was followed between six genes, recombination between two of them, *th* and *st*, was so rare that for all practical purposes they marked a single locus, and so *st* will be omitted from further consideration. The section of the chromosome studied may thus be divided into four regions, as shown below:



The offspring of the backcross were classified as to which, if any, of the above regions of their maternally derived third chromosome had undergone crossing over. The results are summarized in tables 1 and 2, where the data from individual females have been averaged together by periods. The total number of flies involved in the two series is 14,088. As is apparent from table 1, complementary crossover and non-crossover classes of flies were for the most part represented by approximately equal numbers. The complementary classes arising from no crossing over (normal and *h th cu sr* e^s) and from single crossing over in region 4 (*h th cu sr* and e^s) showed the largest deviations from equality. Since such significant viability differences between classes as did obtain were the same in the two series, they cannot be a factor in any inequality in the amount of crossing over observed in the two groups.

Figure 1 summarizes graphically the data of tables 1 and 2. The abscissa of this figure is the time axis of the experiment; the ordinate represents the observed mean frequency of recombination per chromosome recovered or, if the decimal is moved two places to the right, total observed map distance between *h* and e^s. The controls showed an initial high in crossing over, followed by a sharp drop and irregularly sustained low. The curve given by the experimental flies appears to be a "damped" replica of the control curve, with a lower initial frequency of recombination but higher later values. The general shape of the two curves is remarkably similar, even to the minor inflections. From the second to the eighth period there is on the average 13 percent more crossing over in the experimental flies than in the controls.

Two questions at once arise concerning these data. First, of what significance are the differences between successive points on the two curves—that is, in this experiment what is the most probable true relation between age and crossing over, from which the data obtained represent random deviations? Second, are these two curves significantly different from one another?

In order to provide an answer to the first question, two row contingency tables were constructed, comparing the frequency and distribution of

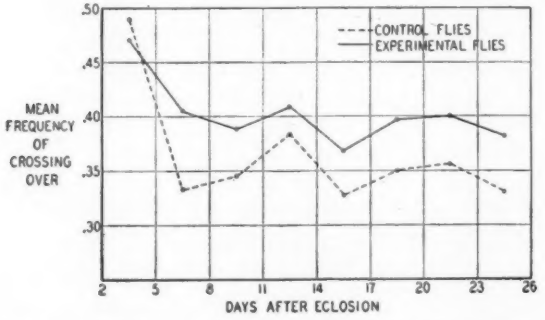


FIGURE 1.—A comparison of control flies and flies developing from partially starved larvae, with respect to the relation between age and the observed mean frequency of recombination in the third chromosome.

crossing over in chromosomes recovered from control flies during successive periods. Thus, a comparison was made of the amount of crossing over observed during the first and second periods, the second and third, the third and fourth, etc. The experimental series was treated in the same way. A χ^2 test of the significance of the differences observed between successive periods was then applied to each table. The analysis revealed that for both series there is a significant difference in the frequency and distribution of crossing over during the first two periods. The only other instance where such a significant difference was noted is between the fourth and fifth periods of control flies. Not only are the successive values observed in the latter seven periods not significantly different, with one exception, but also no clearly defined trend in the data is apparent. Otherwise put, between the fifth and twenty-sixth days of the experiment, the points of the two curves shown in figure 1 do not deviate significantly from a straight line of zero slope. In the case of the control flies, the straight line which best fits the latter seven points would intercept the ordinate at about 0.350, while a similar line of fit to the experimental results would cross the ordinate in the neighborhood of 0.395. In these experiments, then, an initial

high in crossing over is quickly followed by a sustained low. Any assumption of a more complicated relationship seems gratuitous. The relation of these findings to the observations of other workers will be discussed in a later section of the paper.

TABLE 3

Test of the significance of the total difference in frequency and distribution of crossing over in control and experimental flies. Details in text.

PERIOD	1	2	3	4	5	6	7	8	TOTALS
χ^2	4.706	14.958	10.479	11.922	8.216	5.416	2.082	2.928	60.707
Degrees of freedom	7	7	6	6	5	4	4	3	42
P	<.7	<.05	<.2	<.1	<.2	<.3	<.8	<.5	<.03 & >.02

We may now consider the second of the two questions raised above—namely, what is the probability that the differences between the two series are of significance? To answer this, contingency tables similar to those described above were prepared, comparing for each period the frequency and distribution of crossing over in chromosomes recovered from control and experimental flies, and a χ^2 obtained for the difference between the two. Table 3 summarizes the results of the analysis. Differences in the number of degrees of freedom in the various periods are due to the fact that when certain crossover classes were poorly represented (fewer than seven individuals), they were combined with adjacent classes in order to get significant numbers. It is apparent that for only one of the eight periods, the second, are the differences between the two series beyond the accepted level of significance. But since both χ^2 and the degrees of freedom are additive quantities, they may be totaled and an estimate obtained of the significance of the differences observed over the entire course of the experiment. When this is done, the probability that crossing over is the same in the two series is less than 0.03 but greater than 0.02.

This method of analysis fails to take into account the consistency of the difference between the two series—that is, the uniformly higher rate of crossing over in the experimental flies, except for the first period. Our previous analysis has shown that between the second and eighth periods the two curves approximate straight lines of zero slope. It should therefore be permissible, for both the control and experimental series, to combine the data of the latter seven periods and set up a single table comparing the chromosomes recovered from the two groups from the fifth to the twenty-sixth days of the experiment. This treatment is shown in table 4. The probability that the two series are not different during this interval is, according to this method of analysis, less than 0.01.

TABLE 4

A comparison of crossing over in control and experimental flies, during the fifth to twenty-sixth days of adult life.

SERIES	REGION								TOTAL
	0	1	2	3	4	1 3	1 4	OTHERS	
Control	4044	797	81	393	436	81	79	18	5929
Exp'l	3697	848	99	440	458	95	99	25	5761

$\chi^2 = 23.671$ Degrees of freedom = 7 $P < 0.01$

In view of the small number of parental flies used in the derivation of the curves (six control and 11 experimental females), the possibility existed that the differences between controls and experimentals were due to the existence in either or both series of one or several aberrant females showing recombination frequencies quite different from those found in the others. However, analysis of the data for consistency by the usual methods employed in the analysis of variance revealed no evidence for a lack of homogeneity in either the control or experimental females.

In the studies dealing with the relation of recombination to age, extremes of temperature, and X-rays, it was found that the region of the chromosome most susceptible to the effects of these agencies was in the vicinity of the centromere (PLOUGH 1917; MULLER 1926; BRIDGES 1929; MATHER 1939). Clear cut evidence for a similar localization of effect was not found in the present work. During the period of the sustained low, the ratio of experimental to control crossing over in the various regions was as follows: *h-th*, 1.121 ± 0.045; *th-cu*, 1.271 ± 0.171; *cu-sr*, 1.166 ± 0.063; and *sr-e*, 1.119 ± 0.065. This grouping of all the data of the latter seven periods bearing on crossing over in a given region seems justified, since, as is apparent from table 2, during these periods the differences between control and experimental results for any given region were rather consistently in the same direction. All of the ratios are above 1.00, two of the four significantly so. The highest ratio is found in the short *th-cu* region, which contains the centromere, but at the same time this ratio differs less significantly from unity than any of the others. This may in part be due to the statistical methods employed, since there is a relatively larger error in the estimation of the length of a small segment than of a large segment. Accordingly, too literal emphasis should not be placed upon the comparative estimates of significance obtained. None of the ratios differs significantly from any of the others. We may therefore conclude that the increase in crossing over is well distributed along the length of the chromosome

studied, with the possibility of a maximum effect in the region of the centromere not excluded.

Of the possible ways of accounting for this generally distributed increase, two are outstanding: (1) a decrease in the interference between adjacent points of crossing over, or (2) a general increase in the coefficient of crossing over, resulting in proportionately greater numbers of both single and multiple exchange chromosomes. These two explanations are not mutually exclusive; the observed results may be due to a suitable combination of both these factors. If the first possibility obtains to any significant extent, then both coincidence values and the proportion of chromosomes which are the result of multiple crossing over within any two regions should be higher in the experimental than in the control series. Unfortunately for the purposes of this experiment, the regions for which it is feasible to calculate coincidence and the proportion of multiples—namely, regions 1 and 3, and 1 and 4—are on opposite sides of the centromere, and it is well known (and confirmed by these data) that as a rule there is no interference between crossing over on opposite sides of this region. A decision between the two possibilities is thus not practical on the basis of the material at hand. It seems unlikely that decreased interference alone can account for the whole of the effect.

DISCUSSION

To the list of agencies known to influence the frequency of crossing over in Drosophila females should be added still another, the type of larval nutrition. However, the detailed effects of nutrition appear to differ from those of the other agencies, which in turn differ among themselves. Thus, the effects of age, temperature, and irradiation are most strongly apparent —or even sharply localized—in the region of the centromere; nutritional effects appear to differ from all these in that the increase was observed to be more uniformly distributed over the entire section of the chromosome under surveillance. KIKKAWA (1934) has stressed the possibility that any experimentally produced increase in crossing over in the region near the centromere is attended by a compensatory decrease in the more remotely situated regions. This possibility also emerged from the earlier irradiation experiments of MAVOR (1923a, 1923b) and MULLER (1926). The present data offer no support to this hypothesis, but it should be pointed out that crossing over was not followed in the most distal portions of the chromosome. The nutritional effects also differ from those of temperature and possibly irradiation in that the former persist longer than the latter after the cessation of treatment.

SEREBROVSKY (1927) and BERGNER (1928) have published data indicating that the frequency of crossing over is correlated with the length of

larval life; the first investigator obtained a negative correlation and the latter a positive. A reexamination of their data in the light of the present findings suggests that the apparent correlation between the length of the larval stage and the frequency of crossing over may be largely or entirely due to a mutual dependence of these two phenomena on a third variable,

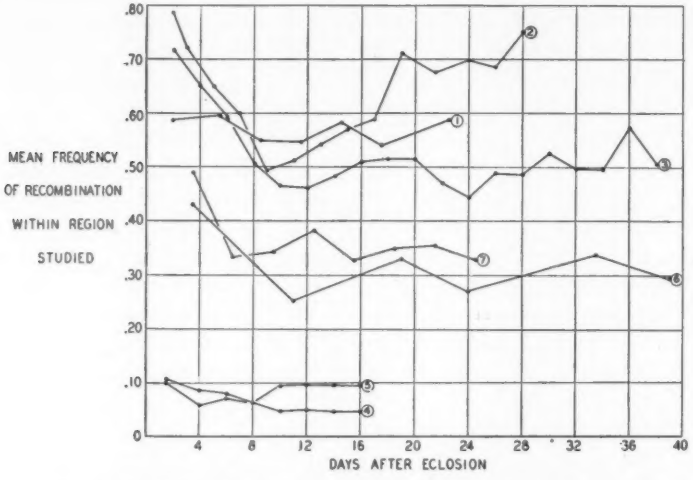


FIGURE 2.—A graphic comparison of the results of five investigators who have studied the relation between age and crossing over in the third chromosome of *Drosophila melanogaster*. The numbers affixed to the various curves refer to the following studies:

Investigator	Region covered
1. PLOUGH, 1921	*se-ss-e^{s}-ro*
2. BRIDGES, 1929, experiment 1	*ru-D-sl-p^{p}-ss-e^{s}*
3. BRIDGES, 1929, experiment 2	*ru-h-D-sl-p^{p}-ss-e^{s}*
4. BERGNER, 1928, control 1	*D-cu*
5. BERGNER, 1928, control 2	*D-cu*
6. POLITZER, 1940	*se-cp-gl^{3}-e''*
7. NEEL, this paper, controls	*h-lh-cu-sr-e^{s}*

a nutritional effect, since the lengthened larval periods in each case were due to poor nutritional conditions. It is not apparent why SEREBROVSKY's results should differ in direction from those of BERGNER and the present investigator.

The work of STADLER (1926) and OEHLKERS and his students and collaborators (cf. OEHLKERS 1937) renders it probable that the amount of crossing over in plants is likewise influenced by nutritional circumstances.

The tendency toward increased crossing over under adverse living conditions is not without adaptive significance to the organism. It would lead to a larger number of new factor combinations under just those conditions

where it is imperative that the organism explore its genetic potentialities to the fullest extent possible.

Finally, we may turn to a consideration of the relation between age and crossing over. This subject has been considered by a number of investigators, and particularly by BRIDGES (1929), KIKKAWA (1934), and POLITZER (1940). These investigators state that the initial high and subsequent decline in the frequency of crossing over are followed by a second, less pronounced high, and a second decline—that is, that with increasing age there are significant periodic fluctuations in the amount of recombination. In figure 2 are summarized graphically the results of five different investigators who have studied under standard conditions the relation between age and crossing over in some portion of the third chromosome of *D. melanogaster*. In examining this collection of curves, one is impressed by two points: (1) the fact that in all cases but one there is an initial high in the frequency of crossing over, followed by a decline, and (2) the considerable degree of divergence in the course of the latter, and numerically less reliable, portions of the curves. An attempt has been made to analyze, insofar as it is possible from the published data, the latter portions of all these curves with respect to possible significant trends, particularly when they are considered in conjunction with one another. This is a rather difficult procedure, but it would appear quite probable that if these curves are all expressions of the same fundamental relationship, then the nature of that relationship in this particular chromosome is an initial high in the frequency of crossing over, followed by a sustained low. Certainly the "periodic fluctuations" can at this stage of the analysis be as well attributed to the operations of chance and uncontrolled environmental variables as to the effect of some inherent physiological rhythm of the female.

Much of this work was carried out during the summer of 1940 at the Department of Genetics, CARNEGIE INSTITUTION OF WASHINGTON, Cold Spring Harbor, New York; I am greatly indebted to the members of this institution for facilities and suggestions.

SUMMARY

The frequency of crossing over in the *h-e*[3] region of the third chromosome of *Drosophila melanogaster* females was significantly increased in flies which during the larval stage had been removed from all food from the seventieth hour of egg-larval life until the time of puparium formation (development at 26°C). Except for the first few days, this increase in the amount of crossing over was consistently apparent throughout the entire 26 day period that the females were under observation.

The increase was distributed along the entire portion of the third chro-

mosome studied; the possibility of a maximum effect in the region of the centromere remains open.

In both the control and the experimental series, the relation of crossing over to age was characterized by an initial high in the frequency of recombination, followed by a sharp decrease and sustained low.

LITERATURE CITED

ALPATOV, W. W., 1930 Phenotypical variation in body and cell size of *Drosophila melanogaster*. Biol. Bull. **58**: 85–103.

BEADLE, G. W., E. L. TATUM, and C. W. CLANCY, 1938 Food level in relation to rate of development and eye pigmentation in *Drosophila melanogaster*. Biol. Bull. **75**: 447–462.

BERGNER, A. D., 1928 The effect of prolongation of each stage of the life cycle on crossing over in the second and third chromosomes of *Drosophila melanogaster*. J. Exp. Zöol. **50**: 107–163.

BRIDGES, C. B., 1929 Variation in crossing over in relation to the age of the female in *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. **399**: 63–89.

GOWEN, J. W., 1919 A biometrical study of crossing over. On the mechanism of crossing over in the third chromosome of *Drosophila melanogaster*. Genetics **4**: 205–250.

KIKKAWA, H., 1934 Studies on non-inherited variation in crossing-over in Drosophila. J. Genet. **28**: 329–348.

LUDWIG, W., 1938 Faktorenkoppelung und Faktorenaustausch bei normalem und aberrantem Chromosomenbestand. Probleme der theoretischen und angewandten Genetik und deren Grenzgebiete. 245 pp. Leipzig: Georg Thieme.

MATHER, K., 1939 Crossing over and heterochromatin in the X chromosome of *Drosophila melanogaster*. Genetics **24**: 413–435.

MAVOR, J. W., 1923a An effect of X-rays on crossing over in Drosophila. Proc. Soc. Exp. Biol. N. Y. **20**: 335–338.

1923b An effect of X-rays on the linkage of Mendelian characters in the first chromosome of Drosophila. Genetics. **8**: 355–366.

MULLER, H. J., 1926 The regionally differential effect of X-rays on crossing over in autosomes of Drosophila. Genetics **10**: 470–507.

NEEL, J., 1941 A relation between larval nutrition and the frequency of crossing over in the adult of *Drosophila melanogaster*. Genetics **26**: 163.

OEHLKERS, F., 1937 Die zytologischen Grundlagen des genetischen "crossing-over." Ber. deuts. bot. Ges. **55**: 96–118.

PLOUGH, H. H., 1917 The effect of temperature upon crossing over in Drosophila. J. Exp. Zoöl. **24**: 147–209.

1921 Further studies on the effect of temperature on crossing over. J. Exp. Zoöl. **32**: 187–202.

POLITZER, O., 1940 Veränderung der Crossoverhäufigkeit durch Einwirkung von Temperatur und Alter. Z. i. A. V. **78**: 129–147.

SEREBROVSKY, A. S., 1927 The influence of the "purple" gene on the crossing-over between "black" and "cinnabar" in *Drosophila melanogaster*. J. Genet. **18**: 137–175.

STADLER, L. J., 1926 The variability of crossing over in maize. Genetics **11**: 1–37.

STERN, C., 1933 Faktorenkoppelung und Faktorenaustausch. Handb. Vererbungsw. 1, H (Lief. 19). vii and 331 pp. Berlin: Gebr. Borntraeger.

THE HOMOLOGIES OF THE CHROMOSOME ELEMENTS IN THE GENUS DROSOPHILA

A. H. STURTEVANT AND E. NOVITSKI
California Institute of Technology, Pasadena, California

Received April 13, 1941

INTRODUCTION

IT IS becoming increasingly clear that certain elements in the chromosome complexes of the Drosophilas maintain their identity from species to species, though undergoing various changes in their relations to each other and in their internal structure (DONALD 1936; STURTEVANT and TAN 1937; STURTEVANT 1938a, 1940). The terminology for these various elements, however, is far from uniform, since several conflicting systems of lettering and numbering are in use. The most satisfactory general solution of the problem of nomenclature seems to be that suggested by MULLER (1940). According to this system the recognizable elements are lettered, in the sequence familiar in *melanogaster*. The X of that species becomes A; IIL, B; IIR, C; IIIL, D; IIIR, E; IV, F. It is not expected that this system will replace those established by long usage, as in *melanogaster*, but it is strongly recommended that it be applied in those cases where the change might more easily be made. Furthermore, arbitrary systems of numbering or lettering used to facilitate studies on the genetics and cytology of unexplored species should be selected with full appreciation of the ambiguities that may arise when the homologies of the various elements become known.

In those cases where species are so closely related that their salivary gland chromosomes show obvious similarities, or that their hybrids can be obtained, the task of homologizing the elements is greatly simplified; for all other species it is necessary to resort to a comparison of their mutant genes with those of species whose elements have been determined. (The term "element" seems the most appropriate one for this unit since chromosome, arm, and limb have all been used with other connotations.) Since only a fraction of the mutants can be regarded as "good" parallels, comparisons must be made with caution; on the other hand, the tendency to minimize otherwise good homologies which do not appear to be consistent with the scheme must be watched carefully.

It is our purpose in the present paper to extend these homologies as far as now seems possible, and to examine critically evidence contrary to the working hypothesis that the elements have remained essentially intact. The species to be so examined include, among others, *affinis*, *algonquin*, *ananassae*, *azteca*, *busckii*, *funebris*, *hydei*, *miranda*, *montium*, *pseudoobscura*, *simulans*, *virilis*, and *willistoni*.

TABLE 1

Compositions of the chromosomes in terms of the elements.

SPECIES	A	B	C	D	E	F	AUTHORITY
melanogaster	X	IIL	IIR	IIIL	IIIR	IV	
affinis	XL	IV	III	XR	II	V	STURTEVANT 1940
algonquin	XS	C	A	XL	B	D	MILLER 1939
ananassae	X part IV part	IIIR	IIIL	IIR	IIL	IV	KIKKAWA 1938
azteca	XS	C	A	XL	B	D	DOBZHANSKY and SOCOLOV 1939
miranda	XL	IV	X_2	XR	II	V	DOBZHANSKY and TAN 1936
pseudoobscura	XL	IV	III	XR	II	V	LANCEFIELD 1922
simulans	X	IIL	IIR	IIIL	IIIR	IV	STURTEVANT 1922
virilis	I	VI	V	III	II	IV	METZ, MOSES and MASON 1923
virilis	V	II	IV	I	III		HEITZ 1934
virilis	X	IV	V	III	II	VI	CHINO 1936
virilis	X	D	E	C	B	M	HUGHES 1939

THE AFFINIS GROUP

The metaphase chromosome configurations of females in this group (*affinis*, *algonquin*, *athabasca*, *azteca*) contain a large pair of V-shaped X chromosomes, two pairs of V-shaped autosomes, a pair of rods, and a pair of microchromosomes (METZ 1916). The original account (DOBZHANSKY in STURTEVANT and DOBZHANSKY 1936) of the salivary gland chromosomes in the members of the *affinis* group has been confirmed and extended by MILLER (1939). The observations of DOBZHANSKY and SOCOLOV (1939) on *azteca* failed to include the longer salivary gland chromosome arm of their "C" chromosome, a circumstance that invalidates their conclusion that "Some genes found in *D. azteca* in a single linkage group must belong to different ones in *D. pseudoobscura;* conversely, some genes linked in *D. pseudoobscura* may be expected to be independent in *D. azteca*."

The salivary gland chromosomes of the different members of the *affinis* group are sufficiently similar so that one familiar with any one of these species can recognize the homologous elements in any other. [Since there is considerable variation in gene sequence within each species, the salivary gland chromosomes of interspecific hybrids from strains taken at random tend to possess quite remotely related chromosomes. These need not be interpreted as interspecific differences, as they have been (BAUER and DOBZHANSKY 1937; DOBZHANSKY 1937), for it is possible that identical or simply related sequences exist within each species.] The homology of the *affinis* elements being known through a comparison of mutant types (STURTEVANT 1940), it was necessary only to correlate the genetic linkage groups with the salivary gland chromosomes of this species. XL of *pseudoobscura* agrees cytologically with the short arm of the X in *affinis* in having

an interstitial heterochromatic region near the base and identical banding at the tip. It follows that the longer arms of the X chromosomes of the two species, containing the same genetic material, are homologous. For the identification of the genetic autosomes with the arbitrarily lettered salivary chromosomes of *azteca* (DOBZHANSKY and SOCOLOV 1939) and of *algonquin* (MILLER 1939), a study was made of nine X-ray induced translocations in *affinis*, with the result shown in table 1. From this correlation it is evident that both elements B and E have median centromeres, yielding the V-shape obvious in two of the metaphase and salivary gland chromosomes.

D. ANANASSAE

The metaphase chromosome configuration of *ananassae* indicates that, while the two large autosomes are not unlike those of *melanogaster*, the centromere of the X chromosome has been shifted from a terminal to a median position and the dot chromosome has been transformed into a small V-shaped chromosome (METZ 1916). The nucleolus-forming region and the bobbed gene, ordinarily related to the sex chromosomes in other species, are associated with the small V-shaped fourth chromosome (KAUFMANN 1937; KIKKAWA 1938). This may have been achieved by a simple exchange of centromeres of the dot and X chromosomes, along with adjacent heterochromatin. If this be the case, the exchange must have occurred prior to the median shift in the centromere of the X; that this sequence is correct is borne out by the cytological observations of KIKKAWA (1936) on *montium*, a relative of *ananassae*, in which the V-shaped fourth chromosome is present, but the X chromosome retains its rod shape. There are other possible explanations, involving an exchange of the Y and fourth chromosome centromeres.

A number of mutants in *ananassae* furnish means for comparing its chromosomes with those of *melanogaster* and with the elements. A more complete discussion of these mutants and their linkage data can, in general, be obtained from the papers of MORIWAKI (1935, 1938) and KIKKAWA (1938). *Melanogaster* mutants with which those of *ananassae* are compared are followed by a symbol showing the limb in which they occur

The X Chromosome

Yellow, scute, white, Notch, cut, singed, miniature, dusky, forked, and Beadex seem good homologies with the same sex-linked mutants of *melanogaster*. The terminology for singed and forked may be reversed, as in *pseudoobscura*, but their value is not thereby impaired, since both occur in the same element. The close linkage of yellow with scute and of miniature with dusky (also found in *melanogaster*, *pseudoobscura*, and *virilis*) adds weight to already good comparisons. Vermilion, ordinarily a good parallel

between the X chromosomes of two species, lacks the confirmation of a feeding or transplantation test.

Chromosome II

Cardinal in the left limb of this chromosome corresponds to cardinal (IIIR) and can be differentiated from its mimics, scarlet and cinnabar, by virtue of its striking change in color with age. Plexate probably represents Delta (IIIR), agreeing both in phenotype and in mutability. Off is much like Bare of *pseudoobscura* (element E), although its dominance, its effect of clipping and removing the bristles, and the occasional viability of the homozygote are not conclusive. Puffed has no homolog in *melanogaster*. but agrees with Puffed in *virilis*, which has small, reddish, and "inflamed" eyes (MORIWAKI 1938). This mutant is located on the second chromosome of *virilis*, which will be shown to correspond to element E. For this reason, we believe that the locus of this gene in *ananassae*, which has not been determined because of its association with an inversion, will prove to be in the left arm.

The right limb of this chromosome has no mutants suitable for homologies, but it may be concluded that it represents the element associated with E in *melanogaster*—that is, D—since elements B and C are both accounted for below.

Chromosome III

Plum, in the left limb of chromosome III, is like Plum (IIR) in *melanogaster* in having a dominant mottled eye color, a recessive lethal effect, and an association with an aberration involving heterochromatin (KIKKAWA 1938). Furthermore, it produces a mottled white eye color in combination with vermilion. This indicates its allelomorphism to brown.

Gap compares with gap (IIR) in weakening the fourth longitudinal vein and in giving the posterior crossvein an oblique direction. This is not conclusive in itself, but serves to confirm the identity of the left limb of the third chromosome of *ananassae* with the right limb of the second chromosome of *melanogaster*.

Only one mutant, plexus, has been found on the right limb of this chromosome. Since we would expect true plexus to occur in the same limb as Plum, we feel that this is in fact net (IIL), one of the most common mutant types in other species.

Chromosome IV

The fourth linkage group contains a dominant mutant, similar to Shaven. This dominant form is not known in *melanogaster*, but is in *simulans* (unpublished data). Haplo-IV individuals correspond quite closely to those of *melanogaster*. The linkage of bobbed with these two has already been discussed.

From the above homologies, it would seem that the nomenclature for the autosomal linkage groups has been reversed with respect to that found in *melanogaster* and, in each case, the significance of left and right as applied to the chromosome arms has also been reversed (table 1).

D. BUSCKII

The metaphase chromosome configuration here is essentially the same as in *melanogaster*, except for the dot chromosomes which are absent as such and may be attached to the X chromosomes as satellites (METZ 1916; KRIVSHENKO 1939). KRIVSHENKO has obtained a considerable number of mutants in this species after treatment with X-radiation. Forked, miniature, Notch, scute, singed, vermilion, white and yellow, all on the X chromosome, may be homologous to the sex-linked mutants in *melanogaster*. Autosomal dominants resulting from X-ray treatment, the most abundant type in KRIVSHENKO's studies, are usually unsuitable for drawing comparisons, partly because of their non-specific effects and partly because of their frequent association with chromosomal aberrations which make linkage studies difficult. One exception in this case is Delta, which occurred seven times, once associated with a IIL–IIIL translocation and once with a IIL–IIR–IIIR translocation, in each case one of the break points occurring in the same region of IIL. This would point to the correspondence of IIL of *busckii* with element E.

D. FUNEBRIS

The mutants in *funebris* available for drawing comparisons are generally unsatisfactory, both because of their indefinite nature and of their unknown linkages, with the exception of a few sex-linked types. Notch corresponds closely to Notch (X) (STURTEVANT 1918). Forked may be either forked (X) or singed (X) (MORGAN, BRIDGES, and STURTEVANT 1925). The similarity of bobbed to bobbed (X, Y) has been pointed out by LUERS (1937). STUBBE and VOGT (1940), by transplantation of eye disks, have shown vermilion to be homologous to vermilion (X). They have also confirmed the identity of autosomal cinnabar with cinnabar (IIR) by the same technique. Radius incompletus bears some resemblances to radius incompletus (IIIL) (TIMOFÉEF-RESSOVSKY 1927) and cubitus incompletus to cubitus interruptus (IV); but any attempt to elaborate these and other possible autosomal homologies seems unprofitable in the absence of linkage data.

D. HYDEI

Notch, white, vermilion, and bobbed in the X chromosome of *hydei* (CLAUSEN 1923; SPENCER 1927) seem probable homologs of the same sex-linked mutants in *melanogaster*. For this species in particular and for many

others there remains unavailable a considerable body of unpublished information, which, as far as we are aware, is not in disharmony with the scheme of permanent elements but which, in a great many cases, furnishes additional evidence in its favor.

D. MIRANDA

The homologies of the salivary chromosomes of *miranda* with those of *pseudoobscura* have been deduced from studies of their hybrids (DOBZHANSKY and TAN 1936). Besides a large number of differences between homologous elements arising, presumably, from the effects of cumulative inversions, DOBZHANSKY and TAN conclude that at least five translocations (involving all the chromosomes) have become established between the two species. In view of MACKNIGHT'S criticism (1939) on cytological grounds, WRIGHT'S mathematical calculations (1940) for the incorporation of one such translocation (see below) and the absence of conclusive evidence to the contrary, we are forced to conclude that these cases very probably do not represent exceptions to the rule of the indivisibility of the elements.

D. MONTIUM

A number of mutants described by OSIMA (1940) provide means for drawing parallels between the linkage groups of montium and the elements. On the X chromosome, white, Notch, and vermilion compare favorably with the similar sex-linked mutants in other species.

Curled-b (left limb of II) simulates curled (IIIR) by curling the wings up and raising and crossing the posterior scutellar bristles. Confluent (left limb of II), obtained twice after X-ray treatment, would correspond to Delta (IIIR), one of the most frequent of mutants found after raying. Hairless, also on chromosome III, agrees with Hairless (IIIR) in its bristle and wing vein diminishing effect, in its dominance and in its lethality when homozygous. This mutant has not yet been allocated to a definite limb; it can be suggested here that it will prove to be on the left limb.

Curled (left limb of III) and jaunty (IIL) seem good parallels; plexus (right limb) would consequently suggest plexus (IIR) rather than net (IIL) since it falls on the anticipated element. On this basis, Plexate, located in the middle of chromosome III, would correspond to Plexate (IIR) and would be expected to occur on the right limb along with plexus. The centromere, therefore, might be expected to fall to the left of Plexate.

The metaphase chromosomes, reported by KIKKAWA and PENG (1938), suggest that this species may be like *ananassae*, but with a terminal instead of a median centromere in the X. The genetic data do not help in checking this supposition.

D. PSEUDOOBSCURA

The metaphase chromosomes of a *pseudoobscura* female consist of a pair of V-shaped X chromosomes, three pairs of autosomal rods, and a pair of dot chromosomes. LANCEFIELD (1922) suggested that a portion of the V-shaped X chromosome of *pseudoobscura* might exist in other species as autosomal material. CREW and LAMY (1935) pointed out, among other comparisons, that certain mutants in the right arm of the X chromosome could be homologized with similar mutants in IIIL of *melanogaster*, although this was not immediately obvious from this paper because of the authors' peculiar terminology. The argument that the elements have remained essentially intact in *pseudoobscura* as compared with *melanogaster* was applied by DONALD (1936) who studied more mutants and implied the necessary corrections to the work of CREW and LAMY. STURTEVANT and TAN (1937) confirmed and extended the main conclusions, leaving no doubt of the identity of the elements in the two species (see table 1). Two additions (STURTEVANT and TAN, unpublished) may be made to the information published in this latter paper: The order of the loci on the X chromosome in the neighborhood of 72 has been shown to be forked, dusky, and bobbed, not dusky, forked, and bobbed as surmised before; also the distance between compressed and sepia is 30 units rather than the indicated 7, thereby increasing all loci from sepia to the right end inclusive by about 25 units.

TAN (1935) correlated the salivary gland chromosomes with the genetic linkage groups in this species.

D. SIMULANS

Since *simulans* hybridizes with *melanogaster*, suspected homologies can be tested directly by mating the corresponding mutants. Thus ordinarily insecure comparisons become unquestionable. The genetic evidence (STURTEVANT 1921a, 1921b, 1929) indicates a close agreement between the limbs of the *simulans* chromosomes and those of *melanogaster* (table 1). The hybrid salivary gland chromosome analyses by PÄTAU (1935) and by KERKIS (1936) confirmed the homologies of the chromosomes and verified the existence of an inverted sequence of genes in the third chromosome, the first inversion to be found in Drosophila (STURTEVANT 1921c). The more detailed examination by HORTON (1939) revealed that as many as 23 additional intra-chromosomal differences, all very small, may exist between the two species.

Six unpublished corresponding mutant types of *simulans* may be noted here: scute (X, 0), ocelliless (X, 24), javelin (III, 0), radius incompletus (III, 58), recessive hairless (III, 61), and dominant Shaven (IV). The sex-

linked parallel vermilion (X, 31.8) was reported by STURTEVANT (1932), without its locus. All of these have been shown to be allelic to the *melanogaster* types whose names they bear and which they closely resemble.

DROSOPHILA VIRILIS

D. virilis remains the only species in the subgenus Drosophila (STURTEVANT 1939) with a sufficient array of mutants to make possible homologizing all the linkage groups with the elements. The six linkage groups agree with the metaphase configuration of five pairs of rods and one pair of microchromosomes. METZ, MOSES, and MASON (1923) and CHINO (1929, 1936) have drawn many comparisons of *virilis* mutants with those of *melanogaster*. *Melanogaster* mutants are followed by a notation of the chromosome limb in which they occur, all others being *virilis* types. We will attempt to evaluate these and, in some cases, make additional suggestions.

X Chromosome

Convincing parallels have been drawn in the cases of yellow, forked, singed, glazed (METZ, et al.), of crossveinless (WEINSTEIN 1920), of scute, white, Notch, miniature, dusky, rudimentary, Beadex, and bobbed (CHINO) with the identically-named mutants (except lozenge for glazed) in the X chromosome of *melanogaster*. HOWLAND, GLANCY, and SONNENBLICK (1937) have established the identity of vermilion of *virilis* with vermilion (X) by the transplantation of eye disks.

The comparisons of echinus with echinus (X), magenta with ruby (X), vesiculated with vesiculated (X), ragged with cut (X), decline with wavy (X), apricot with garnet (X), and small bristle with tiny (X) (CHINO) are open to doubt, but they do serve as corroborative evidence that the composition of the X chromosome of *virilis* and *melanogaster* is essentially the same.

Chromosome II

Confluent is very probably homologous to Delta (IIIR) (CHINO), although the weaker alleles described by METZ et al. show some discrepancies. Concave and crumpled (IIIR) constitute one of the most striking cases of homology (METZ et al.); the case is strengthened by the occurrence of similar types in element E of *affinis* and *pseudoobscura*. Our examination of varnished indicates that it is a good parallel to glass (IIIR).

It has been suggested that ebony corresponds to ebony (IIIR), brick to claret (IIIR), and broken to crossveinless-b (IIIR) or to crossveinless-c (IIIR) (CHINO). These are not diagnostic mutants and may be questioned, although they do fall in the proper limb.

Radius incompletus (IIIL) has been compared to both incomplete and

detached of virilis (CHINO). From the published figures, it would seem that incomplete is quite like radius incompletus (IIIL), but this homology loses its force since it is not in the expected element. A similar situation is found in the case of lanceolate (see below).

Thus chromosome II of *virilis* corresponds to IIIR of *melanogaster*, or element E.

Chromosome III

Short veins suggest veinlet (IIIL) (CHINO) but the comparisons of rose with rose (IIIL), spread with dihedral (IIIL), and rolled with rolled (IIIL) (CHINO) are less convincing.

The striking resemblance of hunch to ascute (IIIL) has been pointed out (METZ et al.) but was discarded as a possible homology because a similar mutant was sex-linked in *D. pseudoobscura*. Since it is now known that the limb of the X chromosome of *pseudoobscura* in which this mutant is located corresponds to IIIL of *melanogaster* (DONALD 1936), this can be considered a convincing parallel. Telescoped has no homolog in *melanogaster*, but agrees closely with deformed and its allele serrate in *willistoni* (LANCEFIELD and METZ 1922) and to compressed in *pseudoobscura* where it is located in element D (STURTEVANT and TAN 1937). Garnet, like Henna (IIIL), is a dominant dark eye color; the only other possible homology would be Plum (IIR), which may be eliminated because of its mottling effect and allelism to brown (see eosinoid below).

The evidence favors the correspondence of *virilis* chromosome III with *melanogaster* IIIL or element D. On this basis, it may be safely predicted that cinnabar, after appropriate tests, will prove to be scarlet (IIIL) and not cinnabar (IIR).

Chromosome IV

Dachsous and dachsous (IIL), Star and Star (IIL) and reduced and reduced (IIL) seem good comparisons (CHINO). Clipped probably represents one of the truncate alleles of dumpy (IIL) (CHINO).

However, CHINO's identification of rough-4a with roughish (IIL), black with black (IIL), Squat with Squat (IIL) and flipper with pupal (IIL) are less convincing. Since the suggested homologs lie in the proper arm, they do tend to confirm the correspondence of these limbs. His figure of veinlet indicates that it is not veinlet (IIIL), as he suggests (see short vein, above).

Plexus might correspond to either net (IIL) or plexus (IIR). We would favor the first alternative since it falls in the proper limb. Lanceolate has been compared to lanceolate (IIR). This is one case where a mutant suitable for homologizing does not have an equally good or better comparison in the anticipated element. The force of this discrepancy is diminished by DONALD'S observation (1936) that a similar mutant is found in element D

in *pseudoobscura*, in addition to that found later in element C (STURTEVANT and TAN 1937). Also, KIKKAWA (1938) lists two such mutants, lance and lanceolate, one on the second, the other on the third chromosome of *ananassae*.

The bulk of the evidence favors the view that the third chromosome is element B.

Chromosome V

Eosinoid agrees with brown (IIR) in giving a white eye color in combination with scarlet or cinnabar (MORI 1937). Vestigial and vestigial (IIR) and straw and straw (IIR) seem excellent homologies (CHINO). Comparisons of ruffled with intwined (IIIL), fat with fat (IIL), dachsoid with four-jointed (IIL), mahogany with clot (IIL) and with sepia (IIIL), Beaded with Beaded (IIIR) and morula with morula (IIR) (CHINO) are questionable. Beaded may be compared with Jagged (C) of *pseudoobscura*.

Branched might be either net (IIL) or plexus (IIR); the latter seems more likely on the basis of the other homologs. For the same reason, we believe that scarlet corresponds to cinnabar (IIR) and not to scarlet (IIIR) (see cinnabar above).

Chromosome VI

The comparison of abdomen rotatum with abdomen rotatum (IV) (CHINO) is convincing; Gap, which shortens the fifth longitudinal vein, may be questioned as a homolog to cubitus interruptus. To these may be added the parallel of stubby with shaven (IV). Thus the dot chromosomes of both species are apparently the same.

As far as the above data can show, the elements have remained essentially intact. We are therefore reluctant to accept in its entirety CHINO's conclusion that "it seems to be justified to assume that in the evolutional course of Drosophila there occurred many inversions, mutual translocations, and attachments or fragmentations of all chromosomes."

FUJII (1936), by means of four translocations and an inversion, has correlated the salivary gland chromosomes with the linkage groups, as indicated in table 1.

SPENCER (1940) has described a native American subspecies, *virilis americana*, in which elements D and E are united to form a V; elements A and B are also fused, the additional B necessary to the diploid complement of the male being present as a single rod (HUGHES 1939; STALKER 1940; PATTERSON, STONE, and GRIFFEN 1940). In another subspecies, *virilis texana*, elements B and D have a single centromere (PATTERSON, STONE and GRIFFEN 1940). In each case the remaining elements are present as rods.

D. WILLISTONI

The X chromosome of this species is V-shaped, as in *pseudoobscura*, while the autosomes are comprised of one V and one rod (LANCEFIELD and METZ 1921). From the metaphase chromosome length relationships, it is clear that one of the elements that is autosomal in most species is part of the X in *willistoni*. The homologies of the sex-linked mutants here are consequently of considerable interest.

Yellow has been compared to yellow (X), scute to scute (X), vermilion to vermilion (X), stubby to forked (X), triple to bifid (X), forked to singed (X) (LANCEFIELD and METZ 1922; FERRY, LANCEFIELD, and METZ 1923). In addition, square agrees with rudimentary (X); unpublished data show that a newly arisen vermilion (locus in X unknown) lacks the v^+ substance, as determined by the feeding technique. Deformed and its allele serrate correspond quite closely to telescope of *virilis* (element D) and to compressed of *pseudoobscura* (element D) (STURTEVANT and TAN 1937); stump compares favorably with radius incompletus (IIIL), and short and veinlet (IIIL) seem good parallels.

The evidence thus indicates that elements A and D have fused to form the X in *willistoni*, as in *pseudoobscura*. This conclusion is strengthened by the distribution of the mutants homologized: all those paralleling element A mutants are found on one half of the X (34.5 to 84) while all those paralleling element D mutants are on the other half (0 to 34). The genetic locus of the centromere on this basis would be between 34 and 34.5. Low interference values for this region, as deduced from the crossing over data of LANCEFIELD and METZ (1922) support this hypothesis.

The autosomal mutants do not yield a satisfactory account of the composition of the V and rod-shaped autosomes. Balloon (chromosome II) has been homologized with balloon (IIR) (FERRY, LANCEFIELD, and METZ 1923). Apterous (chromosome II) agrees well with apterous (IIR); both remove the wings, reduce the balancers, diminish the number of posterior scutellar bristles, deform the thorax, and sterilize both sexes. Clipped and Scalloped, both on chromosome II, probably represent the dominant vestigial deficiencies (IIR). Approximated and dachs (IIL) are close parallels. These homologies indicate that the second chromosome of *willistoni* includes elements B and C, as in *melanogaster*. The location of Knot, which bears some resemblances to Delta (IIIR), on the second chromosome casts doubt on this conclusion. In either case, however, chromosome II must be the autosomal V; this is supported by the slightly greater number of mutants in II than in III (11 in II as compared to 8 in III).

Neither the genetic nor the cytological evidence gives a clue as to the location of element F in *willistoni*, but it is to be noted that the salivary

gland chromosomes have not yet been studied in this species. The most likely place to look for F would appear to be as a short arm on the apparently rod-shaped chromosome (III, element E?).

HOMOLOGOUS GENES IN THE VARIOUS SPECIES

Table 6 lists the homologous genes in the species discussed, to which some previously unpublished cases have been added. Unpublished data of other workers, available particularly in Drosophila Information Service, tend to strengthen the suggested homologies given in the table.

The occurrence of "sex-ratio" in *melanica*, a member of the subgenus Drosophila, seems worthy of special mention. It has been previously noted only in the subgenus Sophophora (*obscura*, *pseudoobscura*, *affinis*, *athabasca*, and *azteca*) and in those cases analyzed, is located in element D, which is part of the X chromosome. It seems likely, then, that the X of *melanica* is a V, one arm of which is element D. The cytological observations of METZ (1916) indicate that a V-shaped chromosome of the required size is present, although no identification of the X has been made.

LENGTHS OF THE ELEMENTS IN THE SALIVARY GLAND NUCLEI

The percentage each element comprises of the total euchromatic length of the salivary gland chromosomes is listed in table 2. These values have been derived from the published lengths for *ananassae*, *melanogaster*, and *virilis*, from measurements of the published drawings for *algonquin* and *azteca*, and from a combination of two sets of drawings plus our estimate for element D for *pseudoobscura*. The figure given for element B of *azteca* has been obtained by assuming that it bears the same ratio to the other autosomal elements as do the homologous elements in *algonquin*.

TABLE 2

Relative lengths of the elements in the salivary gland nuclei.

	A	B	C	D	E	AUTHORITY
algonquin	16.4	18.5	19.7	20.0	25.4	MILLER 1939
ananassae	19.3	19.1	18.0	18.4	25.1	KIKKAWA 1938
azteca	15.1	18.8	19.0	21.4	25.8	DOBZHANSKY and SOCOLOV 1939
melanogaster	18.9	18.5	21.0	18.0	23.6	BRIDGES 1935
pseudoobscura	14.0	19.9	16.3	24.0	25.8	TAN 1936; DOBZHANSKY and TAN 1939
virilis	19.3	19.3	19.5	18.3	23.5	FUJII 1936
virilis	19.3	20.1	19.0	18.2	23.4	HUGHES 1939

Since these lengths are a function of both the elasticity of the chromosomes and the personal equation of the observer, the comparative lengths of the homologous elements are quite variable, with one striking exception: in every case element E is considerably longer than any of the others. This

identification of the longest salivary gland element with element E may be of practical value in those instances where the correlation may be impracticable for other reasons. SLIZYNSKA and SLIZYNSKI (1941) report that in *funebris*, one of the elements is considerably longer than any of the others; it therefore probably corresponds to element E.

It is also to be noted that element D is about the same length as A when it is autosomal, but is distinctly longer in the three species in which it is part of the X. It seems clear from the table that the lengths of element A and D tend to show a negative correlation with each other. Numerous speculations are suggested by these relations, but they can scarcely be profitably discussed without more information than is now available.

SEQUENCE OF CORRESPONDING GENES IN DIFFERENT SPECIES

In general there is little similarity in the sequence of corresponding loci within each element, except when such closely related species as *melanogaster* and *simulans* are compared. In discussing *melanogaster* and *pseudoobscura*, STURTEVANT and TAN (1937) state: "The mathematical properties of series of letters subjected to the operation of successive inversions do not appear to have been worked out, so that we are so far unable to present a detailed analysis. It does appear, however, that the five arms (taken together) are definitely more alike in the two species than could result from chance alone." These statements now require some modification.

With the help of PROF. MORGAN WARD, a beginning has been made in the study of the mathematical consequences of successive inversions. Complete catalogs have been prepared, showing all the possible different arrangements of 2, 3, 4, 5, and 6 loci, respectively, together with the minimum number of successive inversions required to change each arrangement into a single arbitrarily chosen one. Actually, numbers were used, and the required arbitrary sequence was the ordinal one (1, 2, 3, 4, etc.). Table 3 shows the mean number of inversions required, together with the standard deviations for the respective populations.

TABLE 3

Mean number of inversions required to transform random arrangements of numbers into ordinal series.

NUMBER OF LOCI	MEAN	STANDARD DEVIATION
1	0	0
2	.500	.50
3	1.167	.69
4	1.750	.66
5	2.392	.70
6	3.036	.71
8	4.367 ± .092	.71 ± .06
9	4.975 ± .13	.82 ± .09

In the cases where more than six loci were involved, it became impracticable to make complete catalogs. Accordingly, for the two rows shown (eight and nine loci), random series of numbers (60 and 40 sequences, respectively) were chosen, and for each such sequence there was determined the minimum number of successive inversions required to reduce it to the ordinal sequence chosen as "standard." For numbers of loci above nine the determination of this minimum number proved too laborious, and too uncertain, to be carried out.

The table as it stands, however, gives a solution that seems safe to use for any numbers of corresponding loci likely to be encountered in such studies as these, since a plot of the values shows that there is an approximately linear relation between the number of loci considered and the average number of inversions required to reduce to ordinal sequence. For each additional locus considered, there is an increase of about .64 in the average number of inversions required. The curve is more irregular near its point of origin; and the values are exact up to six loci. Accordingly, for values above six it seems safest to start with the value 3.036 (for 6) and add to it $.64(n-6)$, where n is the number of loci concerned.

The standard deviations shown in the table give a less regular curve when plotted, but it is clear that they are increasing only slowly. It may probably be assumed that σ will not be greater than 1 for values up to $n = 15$—beyond which it is unlikely that the present problem will require a solution for many years. This means that the spread is not great—in other words, that any random sequence is unlikely to require a number of inversions much different from the calculated one. If a number much less is in fact encountered, it may be concluded that there is a significant degree of resemblance. This method has been applied to the sequence differences between *melanogaster* and *pseudoobscura*, with the result shown in table 4. Evidently the two species are not more alike than could easily result from chance alone.

TABLE 4

Comparison of the required and calculated numbers of inversions to change the melanogaster into the pseudoobscura sequences.

ELEMENT	A	B	C	D	E	TOTAL
Loci	13	6	6	6	7	
Inversions required	7	2	4	3	3	19
Inversions calculated	7.6	3.0	3.0	3.0	3.7	20.3

In any series of successive loci, each locus has two neighbors, and each of these may be either "right" or "wrong"—that is, may or may not be one that lies adjacent to the given locus in the arbitrarily chosen standard

ordinal sequence. For a terminal locus there is only one adjacent locus, but the terminal position itself may be taken as constituting a "connection" and then may be treated in the same way, a "right connection" here meaning that the terminal locus is not only terminal in the chosen standard sequence but also lies at the same end (proximal or distal). There are then n+1 "connections" in any sequence of n loci. It may be shown that in any series of random arrangements the average number of "right" connections is two, regardless of the value of n. It is also evident that any single inversion changes two and only two connections, since it has two ends, each of which must fall in a connection. These two relations are very helpful in working out the consequences of successive inversions. The conception of "right connections," however, is responsible for the incorrect conclusion drawn by STURTEVANT and TAN. One of the right connections appearing in their comparison has now disappeared, as a result of the revision of the *pseudoobscura* sequence recorded above (it may be noted that this revision does not change the number of inversions required to transform one sequence into the other). The result now is that there are 13 "right" connections (that is, identical in the two species) where ten are expected (two in each of the five elements) on chance alone. The difference is probably not a significant one.

This analysis does not take into account the distances concerned—a connection may be assumed to be like in two elements regardless of the amount of crossing over shown. Thus, in element B, jaunty and hook are adjacent in the series of corresponding genes both in *melanogaster* and in *pseudoobscura;* but the crossover values are 5.2 and 23.1, respectively. In such a case, it is probable that further corresponding loci will show that the sequence resemblance is an accidental one. For certain pairs of loci that lie quite close together, the situation is different, as may now be shown.

Yellow and scute are quite near each other in *melanogaster*, *simulans*, *pseudoobscura*, *virilis*, *ananassae*, and *willistoni;* they are separated by other loci in *affinis*. (Unpublished data of DR. W. P. SPENCER indicate that they are also separated in *hydei*.) Notch and white are from one to four units apart in *melanogaster*, *simulans* (Notch is not known here, but facet serves to identify its locus), *ananassae*, *montium*, *hydei*, *virilis*, and *pseudoobscura*. Miniature and dusky give few if any crossovers in *melanogaster*, *ananassae*, *pseudoobscura*, and *virilis*. It may be noted that these are all short distances, not only in terms of crossing over, but also in terms of the salivary gland chromosome maps of *melanogaster*, which is the only species for which element A is adequately known in the salivary glands.

It may be concluded that inversions with end-points falling within these short sections have not become established during the differentiation of

these species, except for those between yellow and scute in *affinis* and in *hydei*. It may be observed that all three sections appear to exist intact in both of the subgenera studied, since *virilis* and *hydei* belong to Drosophila while all the other forms named are members of the subgenus Sophophora. Evidently, then, these sections represent associations of loci that have been in their present conditions for a very long period of time.

DISCUSSION

The essential argument showing the improbability of the incorporation of a translocation into a population has been published a number of times and has been prevalent in genetic thought since an earlier date; nevertheless, it seems opportune to recapitulate it briefly here, with specific reference to the Drosophilas.

Translocations have been observed to occur spontaneously under laboratory conditions (all the Drosophila translocations discovered prior to the advent of the X-ray technique fall in this category) and, in the few cases mentioned below, have been found in natural populations. It seems not unreasonable to assume therefore that they continually arise in nature with some very low frequency. Once having arisen, such a translocation would exist in the heterozygous state and thereafter chiefly in the heterozygous rather than homozygous state. The extensive studies on such rearrangements in Drosophila have amply demonstrated the selective disadvantage which their heterozygotes must suffer as a consequence of their production of unbalanced gametes. Thus a certain number of translocated chromosomes along with a number of normal chromosomes is lost in every generation; the percentage loss of the former may be considerable because of its low total frequency, whereas the percentage loss of the latter is usually insignificant. In this way selection discriminates against the less prevalent arrangement, progressively decreasing its frequency until it is wiped out.

It follows that the sole opportunity for a translocation to become established lies in its attaining a percentage frequency sufficiently great so that the discrimination of selection is not adequately expressed before chance changes the ratio of the frequencies in favor of the newer arrangement, whereupon the original is eliminated. A high percentage frequency can be reached only when the total number of chromosomes is low—that is, when the population size (N) is small. Furthermore, the population size must be small for chance to upset the ratio of the two arrangements in favor of the newer type.

For reciprocal translocations in which only the completely balanced types survive, WRIGHT (1940) has shown that fixation is difficult unless N is very low. Certain types of translocations might be expected not to

encounter such drastic adverse selection, by virtue of the viability and fertility of individuals possessing an unbalanced (that is, heterozygous for a duplication or a deficiency or both) complement of chromosomes (STURTEVANT 1938b). The effect of this condition is to decrease the selection against the new arrangement and so to increase the maximum population size into which it might be incorporated. PROFESSOR WRIGHT has given us his kind permission to present here some more exact probabilities which he has calculated as an extension to his previous statements (WRIGHT 1940). For a translocation whose unbalanced products are completely inviable, the probabilities of fixation are of the order of 10^{-3} if $N=10$, 2×10^{-6} if $N=20$, and 3×10^{-14} if $N=50$. In the most favorable case, when the heterozygous unbalanced products are both viable and fertile (only the homozygous deficiencies being eliminated), the probabilities are roughly 3×10^{-3} in populations of 20, 4×10^{-6} in populations of 50, and 3×10^{-10} in populations of 100. For the case of a small insertional translocation whose homozygous duplication product is not always accompanied by a homozygous deficiency and consequently may be viable and fertile, the probabilities must be somewhat greater than those given for the last case. Nevertheless, they are still very small and cannot greatly increase the maximum values indicated for N in the last case.

The various factors involved in the incorporation of a translocation into a population may be summarized as follows:

1. The translocation must occur in at least one of the individuals of a subpopulation. As N becomes smaller, the probability of this event becomes smaller, the relation being an approximately linear one.

2. The translocation must become incorporated into the whole subpopulation. N must be very small (see figures above); as N increases, the probability decreases rapidly.

3. The subpopulation must not become extinct. As the value of N increases, the probability that the subpopulation will survive increases.

4. The translocation must maintain itself in the descendants of the subpopulation, in competition with normal chromosomes that may be introduced by interbreeding with other subpopulations. As N increases, this probability also increases, since each introduced chromosome has a smaller effect on the composition of the subpopulation.

The third factor might be omitted, since it could be treated merely as decreasing the total number of subpopulations concerned. It is included here to emphasize the point that, if there is variability in the size of the subpopulations, the smaller ones are more likely to become extinct than the larger ones.

The final probability of the establishment of a translocation is the product of the four probabilities just enumerated. The first, third, and

fourth components increase with an increase in N, the second decreases very rapidly with an increase in N. The result is that the product of the four must always be very small. The only situation in which the establishment of a translocation seems likely to occur with any considerable frequency—even if a long period of time be assumed—is that in which hermaphroditic individuals frequently self-fertilize—that is, when there are many subpopulations in which $N = 1$. It would thus seem desirable to have suspected cases of translocation in Drosophila supported by more conclusive evidence than has been considered necessary in the past.

For a translocation to be observed in a natural population, it must be found in the relatively short interval between its origin and its elimination. It seems significant that, despite the extensive genetic and cytological analyses of natural populations of Drosophila, not one translocation has been reported.

In the grasshopper Trimerotropis, CAROTHERS (1931) found a single individual heterozygous for a translocation among fifteen specimens from one locality. A more substantial case from the population standpoint is that reported by WHITE (1940). In 1934 he found a specimen of the grasshopper Metrioptera heterozygous for a translocation and in 1937 recognized the same translocation in some more individuals from the same locality. We are not familiar with the genetic properties of this translocation, with the statistical data for this case, or with the population mechanics of this genus; it therefore seems unwise to discuss the situation further.

Among hermaphroditic plants there are many instances of translocations that have become established. Here, however, the population size may frequently reach the theoretical minimum value of one and remain essentially that for several generations.

A class of "neutral" translocations which might be expected to give non-random segregation in the heterozygote includes those found in *virilis americana* and *virilis texana*, where two elements, ordinarily separate, have fused near the centromere. Such a fusion would seem likely to result, in the heterozygote, in almost completely regular disjunction of the individual rods from their attached homologs. If, in this particular case, there is some slight irregularity in disjunction, it would seem to be more than compensated for by the small sizes of the populations in which these two subspecies are found (PATTERSON, STONE, and GRIFFEN 1940).

RHOADES (1940) has demonstrated the instability inherent in a telocentric chromosome in maize; both the X and fourth chromosomes of *melanogaster*, once considered to have terminal centromeres, have been shown to have two arms (KAUFMANN 1934; GRIFFEN and STONE 1939, 1940). The view of NAWASCHIN (1916) and LEWITSKY (1931) that telo-

centric chromosomes do not exist is considerably strengthened. On this basis, apparent rods in Drosophila have a minute heterochromatic second arm. This arm would then serve as an anchor on which another element, less its centromere, could become attached by translocation, forming a V.

The reverse change, the formation of two rods from a V, demands an additional centromere. STURTEVANT and TAN (1937) suggest that "perhaps the spindle attachment of the Y chromosome is somehow utilized, since the properties of this chromosome allow it to be present as an extra or a fragment without damage to the organism." Means are thus provided for increasing the chromosome number. The one clear instance where such an increase has occurred, in *obscura* Gershenson, involves the breakage of a V-shaped element into two rods.

From the genetic data and metaphase chromosome configurations in *melanogaster*, *willistoni*, *immigrans*, *virilis*, and *pseudoobscura*, it is apparent that they all differ in the associations of their elements. Regardless of the metaphase chromosome configuration that may be assumed ancestral to that of all the Drosophilas, both fusion and fragmentation must have taken place. Although not in complete agreement with the systematic relationships, perhaps the simplest interpretation is that the ancestral type corresponded to the *virilis* configuration, five pairs of rods and a pair of dots. With slight modifications, mostly in the distribution of heterochromatin, this is the most common in the subgenus Drosophila. All other types can be derived by simple fusions of elements and changes in the positions of the centromeres within the elements by inversions, with the exception of *obscura* Gershenson and *ananassae*, as noted above. Within *virilis* itself, a single translocation of this kind has evidently given rise to the configuration found in subspecies *texana;* and two translocations from the *texana* arrangement have produced that of subspecies *americana*. The sequences found within the elements are in agreement with this conclusion (PATTERSON, STONE, and GRIFFEN 1940).

It seems clear that, in general, decrease in chromosome number is more easily brought about than is increase. Accordingly lower chromosome numbers may be regarded as probably (though not necessarily in every case) more recent than higher numbers. This conclusion will hold, however, only in forms—such as Drosophila—in which chromosomes frequently have nearly terminal centromeres, with one arm made up of heterochromatin.

A NOTE ON THE RELATIONS BETWEEN THE SPECIES GROUPS OF SOPHOPHORA

It was suggested by STURTEVANT (1940) that the *willistoni* (AD, BC, EF) and *melanogaster* (A, BC, DE, F) chromosome configurations have been derived from the type now found in *pseudoobscura* (AD, B, C, E, F).

If this be so, the first event was probably the union of elements B and C, to give a hypothetical arrangement AD, BC, E, F. From this, a union of E and F presumably gave the *willistoni* type, while a translocation between AD and E gave the *melanogaster* one.

The hypothetical arrangement, which seems likely to have existed even if the sequence of events was different from that outlined above, has not been reported. In the absence of the identification of the X, it would be listed as the *melanogaster* type; accordingly we have been led to examine the descriptions of the phenotypic characters of the few Sophophoras for which such a report exists. One of them, *D. suzukii*, turns out in fact to be intermediate between the *melanogaster* and *obscura* species groups, as shown in table 5. We are inclined to surmise that this species (recorded by KIKKAWA and PENG (1938) from Japan and China) will be found to have the hypothetical configuration.

TABLE 5

Comparison of some phenotypic characters of the melanogaster and obscura species groups and D. suzukii.

	COLOR	COSTAL INDEX	4TH VEIN INDEX	SECOND ORAL	MIDDLE ORBITAL
melanogaster group	yellow	1.2–2.2	2.2–2.7	long	short
suzukii	yellow	4.0*	2.2	long	long
obscura group	black	2.7–3.0	1.7–2.1	short	long

* The description given by KIKKAWA and PENG lists the costal index as 4.0; their illustration (Plate 32) suggests that this is perhaps a misprint for 3.0, which would make *suzukii* fall within the range of variation of the *obscura* species group.

THE EFFECT OF PERICENTRIC INVERSION ON THE ELEMENTS

None of the changes discussed above interferes with the integrity of the elements. There remains, however, one mechanism which may exchange genic material from one element to another. If two elements are united to form a V, an inversion across the centromere (pericentric) will shift genes from one to the other, and *vice versa*. Heterozygotes for this type of inversion are at a selective disadvantage because single crossovers within the inverted section produce inviable duplication-deficiency zygotes, thus leading to a situation similar to that of translocations. However, if crossing over be hampered, either by the nature of the inversion or by the presence of a crossover suppressor, the new arrangement may become established in the population. The products of a subsequent separation of these two limbs will simulate the effects of a reciprocal translocation.

MILLER (1939) has recorded such an inversion in *algonquin*, occurring with a high frequency in eight out of nine localities studied between Quebec, Canada, and Wooster, Ohio. In this case, only one element (E) was

TABLE 6

Summary of the corresponding mutant types in the different species.

ELEMENT A

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
yellow	yellow	yellow	yellow	yellow	yellow	***buschii, willistoni***
scute	scute	scutellar	scute	scute	scute	***buschii, takahashii, willistoni***
prune	prune					
white	white	white	white	white	white	***buschii, kydei, montium***
facet	facet					
Notch		Notch		Notch	Notch	***buschii, funebris, kydei,***
echinus		echinus		echinus?		***[montium***
bifid						***willistoni*** (triple)
ruby	ruby			magenta?		
crossveinless	crossveinless			crossveinless		
vesiculated	vesiculated			vesiculated?		
cut	cut	beaded	cut	ragged?	cut	
singed	singed	forked		singed	singed?	***buschii, willistoni*** (forked)
ocelliless	ocelliless					
lozenge	lozenge	glazed		glazed, rugose		***[montium? williistoni***
vermilion	vermilion	vermilion	vermilion	vermilion	vermilion?	***buschii? funebris, kydei?***
miniature		miniature	miniature	miniature	miniature	***buschii, takahaskii***
dusky	dusky	dusky		dusky	dusky	***algonquin***
garnet	garnet			apricot?		
rudimentary	rudimentary			rudimentary		***willistoni*** (square)
forked	forked	singed		forked	forked?	***buschii, funebris? willistoni***
Beadex		Pointed		Beadex	Beadex	***[***(stubby)
fused	fused					
bobbed	bobbed	bobbed	bobbed	bobbed	on chrom. IV	***kydei, funebris***

ELEMENT B

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
dachsous				dachsous		
net		tangled	net	plexus	plexus	
Star		Rough		Star		
Curly		Curly				
dumpy	Truncate		truncate	Clipped		
dachs						***willistoni*** (approximated)
abrupt		incomplete				
black	black			black?		
jaunty		jaunty	jaunty			***montium*** (curled)
reduced				reduced		
hook		hook				

ELEMENT C

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
straw	straw		straw	straw		
apterous						***willistoni***
cinnabar		orange	cinnabar	scarlet?		***athabasca, algonquin, funebris***
vestigial	vestigial-nick	Jagged		vestigial		***willistoni*** (Clipped, Scalloped)
gap					gap	
curved		curved				
arc	arc					
plexus		plexus		branched?		***montium***
brown		purple		eosinoid		
Plum					Plum	
lanceolate		narrow	narrow			
	polychaete	polychaete, Scute				

ELEMENT D

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
veinlet		short	veinlet	short veins		*willistoni* (short)
javelin	javelin	slender				
Henna				Garnet		
sepia	sepia	sepia				
hairy			hairy			
tilt		tilt	tilt			
scarlet	scarlet	scarlet	scarlet	cinnabar?		
ascute		ascute	ascute	hunch		
r. incompletus	r. incompletus	snapt				*willistoni* (stump)
		compressed		telescoped		*willistoni* (deformed, serrate)
		sex-ratio	sex-ratio			*athabasca, azteca, melanica, obscura*

ELEMENT E

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
pink	peach	pink, claret?	pinkish, claret?			
curled		upturned				*montium* (curled-b)
Stubble		Stubble				
bithorax		bithorax	bithorax			*athabasca*
aristapedia	aristapedia	aristapedia				
glass		glass		varnished		
Delta	Delta	Smoky	Delta	Confluent, Delta	Plexate	*busckii, montium* (Confluent)
Hairless	hairless					*funebris, montium*
cardinal		cinnabar			cardinal	*azteca*
crumpled		crumpled	crumpled	concave		
claret	claret	claret, pink?	pinkish, claret?	brick?		
		Bare			Off?	
		pauciseta	pauciseta			
				Puffed	Puffed	

ELEMENT F

melanogaster	*simulans*	*pseudoobscura*	*affinis*	*virilis*	*ananassae*	other species
abdomen rotatum			abdomen rotatum	abdomen rotatum		
grooveless		grooveless				
shaven	Shaven		reduced?	stubby	Shaven	
Minute-4	Minute-4					

involved (this element being a V in this species), and another associated inversion acted as a crossover suppressor. The fact that this inversion has been found to have a high frequency makes more probable the occurrence of similar inversions in other species where a V is composed of two different elements.

It is self-evident that the scheme of the integrity of the elements must break down for the more distantly related species of the Diptera. Where this will first happen is not clear. The common metaphase chromosome configuration among the higher Diptera has six chromosomes; whether these are the Drosophila elements with a few alterations or whether they represent quite different gene combinations can be answered only after a more thorough analysis of their genetics is made.

SUMMARY

The six chromosome arms of *D. melanogaster* (X, IIL, IIR, IIIL, IIIR, IV) retain their essential identity among the species of Drosophila so far studied. They are here called "elements" and are designated by the letters A to F in the order just stated.

The corresponding mutant genes on which this conclusion is based are summarized in table 6 of this paper; and the relation of the letter designations to the various chromosome terminologies previously used is summarized in table 1.

Element E is consistently the longest of the six, when studied in the salivary gland chromosomes; element F is always very short. The others are so nearly the same in size that their lengths are not diagnostic.

Analysis of the mathematical properties of sequences subjected to repeated inversions indicates that, in the specific case of *melanogaster* and *pseudoobscura*, the sequences are not significantly more alike than might result from chance.

Three pairs of loci—yellow and scute, Notch and white, miniature and dusky—apparently represent sections which have remained intact over a long period, since the two members of each pair are associated in numerous species and in representatives of both subgenera studied. Yellow and scute are separated, however, in *affinis* and in *hydei*.

The conclusion that the elements remain intact within the group means that few or no translocations have become established in the history of these two subgenera. Analysis of the conditions necessary for such establishment leads to the conclusion that the supposed instances of its occurrence as between species are not adequately established—with the exception of the attachment of part of element A to element F in *ananassae*.

The various ways in which the elements are attached to each other may be supposed to result from a special type of translocation in which both breaks occur in heterochromatin near the centromeres. Under the conditions found in most Drosophila species, this type of translocation more easily decreases chromosome number than increases it. It may be surmised that the configuration found in *virilis* (where all six elements are separate) is the most primitive one among those yet analyzed in Drosophila.

LITERATURE CITED

BAUER, H., and T. DOBZHANSKY, 1937 A comparison of gene arrangements in *Drosophila azteca* and *D. athabasca*. Genetics **22**: 185.

BRIDGES, C. B., 1935 Salivary chromosome maps. J. Hered. **26**: 60–64.

CAROTHERS, E. E., 1931 The maturation and segregation of heteromorphic homologous chromosomes in Acrididae (Orthoptera). Biol. Bull. **61**: 324–349.

CHINO, M., 1929 Genetic studies on the Japanese stock of *Drosophila virilis*. Report 1. Japan. J. Genet. **4**: 117–131.

1936–1937 The genetics of *Drosophila virilis*. Japan. J. Genet. **12**: 187–210, 257–277; **13**: 100–120.

CLAUSEN, R. E., 1923 Inheritance in *Drosophila hydei*. Amer. Nat. **57**: 52–58.

CREW, F. A. E., and R. LAMY, 1935 Linkage groups in *Drosophila pseudoobscura*. J. Genet. **30**: 15–29.

DOBZHANSKY, T., 1937 Genetics and the origin of species. 364 pp. New York: Columbia Univ. Press.

DOBZHANSKY, T., and D. SOCOLOV, 1939 Structure and variation of the chromosomes in *Drosophila azteca*. J. Hered. **30**: 3–19.

DOBZHANSKY, T., and C. C. TAN, 1936 Studies on hybrid sterility. III. A comparison of the gene arrangements in two species, *Drosophila pseudoobscura* and *Drosophila miranda*. Z. i. A. V. **72**: 88–114.

DONALD, H. P., 1936 On the genetical constitution of *Drosophila pseudoobscura*, race A. J. Genet. **33**: 103–122.

FERRY, R. M., R. C. LANCEFIELD, and C. W. METZ, 1923 Additional mutant characters in *Drosophila willistoni*. J. Hered. **14**: 372–384.

FUJII, S., 1936 Salivary gland chromosomes of *Drosophila virilis*. Cytologia **7**: 272–275.

GRIFFEN, A. B., and W. S. STONE, 1939 The demonstration of a new chromosome arm in *Drosophila melanogaster*. Genetics **24**: 73.

1940 Studies in the genetics of Drosophila. IX. The second arm of chromosome 4 in *Drosophila melanogaster*. Univ. Texas Pub. No. **4032**: 201–207.

HEITZ, E., 1934 Über α- und β-Heterochromatin sowie Konstanz und Bau der Chromomeren bei Drosophila. Biol. Zbl. **54**: 588–609.

HORTON, I. H., 1939 A comparison of the salivary gland chromosomes of *Drosophila melanogaster* and *Drosophila simulans*. Genetics **24**: 234–243.

HOWLAND, R. B., E. A. GLANCY and B. P. SONNENBLICK, 1937 Transplantation of wild type and vermilion eye disks among four species of Drosophila. Genetics **22**: 434–442.

HUGHES, R. D., 1939 An analysis of the chromosomes of the two subspecies *Drosophila virilis virilis* and *Drosophila virilis americana*. Genetics **24**: 811–834.

KAUFMANN, B. P., 1934 Somatic mitoses of *Drosophila melanogaster*. J. Morph. **56**: 125–155.

1937 Morphology of the chromosomes of *Drosophila ananassae*. Cytologia (Fujii Jubilee Volume) 1043–1055.

KERKIS, J., 1936 Chromosome configuration in hybrids between *Drosophila melanogaster* and *Drosophila simulans*. Amer. Nat. **70**: 81–86.

KIKKAWA, H., 1936 Two races of *Drosophila montium*. Japan. J. Genet. **12**: 137–142.

1938 Studies in the genetics and cytology of *Drosophila ananassae*. Genetica **20**: 458–516.

KIKKAWA, H., and R. T. PENG, 1938 Drosophila species of Japan and adjacent localities. Japan. J. Zool. **7**: 507–552.

KRIVSHENKO, Y. D., 1939 A comparative genetic study on *Drosophila busckii* and *Drosophila melanogaster*. Acad. Sci. Ukrainian SSR, Inst. Zool., Memoirs on Genetics **3**: 15–89.

LANCEFIELD, D. E., 1922 Linkage relations of the sex-linked characters in *Drosophila obscura*. Genetics **7**: 335–384.

LANCEFIELD, R. C., and C. W. METZ, 1921 Non-disjunction and the chromosome relationships of *Drosophila willistoni*. Proc. Nat. Acad. Sci. **7**: 225–229.

1922 The sex-linked group of mutant characters in *Drosophila willistoni*. Amer. Nat. **56**: 211–241.

LEWITSKY, G. A., 1931 The morphology of the chromosomes. Bull. Appl. Bot. **27**: 19–173.

LUERS, H., 1937 Ein Beitrag zur vergleichenden Genetik an Hand des dominanten Bobbed im Y-Chromosome von *Drosophila funebris*. Z. i. A. V. **74**: 70–90.

MACKNIGHT, R. H., 1939 The sex-determining mechanism of *Drosophila miranda*. Genetics **24**: 180–201.

METZ, C. W., 1916 Chromosome studies on the Diptera. II. The paired association of chromosomes in the Diptera, and its significance. J. Exp. Zool. **21**: 213–279.

METZ, C. W., M. S. MOSES, and E. D. MASON, 1923 Genetic studies on *Drosophila virilis* with considerations on the genetics of other species of Drosophila. Carnegie Inst. Wash. Pub. **328**, 94 pp.

MILLER, D. D., 1939 Structure and variation of the chromosomes in *Drosophila algonquin*. Genetics **24**: 699–708.

MORGAN, T. H., C. B. BRIDGES, and A. H. STURTEVANT, 1925 The genetics of Drosophila. Bibliogr. Genet. **2**: 1–262.

MORI, K., 1937 A study on the development of pigments of various eye color mutants of Drosophila. Japan. J. Genet. **13:** 81–99.

MORIWAKI, D., 1935 Some mutant characters in *Drosophila ananassae*. Genetica **17:** 32–46.

1938 The genetics of some mutant characters in *Drosophila ananassae*. Japan. J. Genet. **14:** 1–22.

MULLER, H. J., 1940 Bearings of the Drosophila work on systematics. The New Systematics, 185–268. Oxford: Clarendon Press.

NAWASCHIN, S., 1916 Sur quelques charactères de l'organisation interne des chromosomes. Rec. d'Art. Sci. dédié à C. Timiriaseff. Moscow. 185–214.

OSIMA, T., 1940 Cyto-genetic studies of *Drosophila montium*. (Report 1.) Cytologia **10:** 450–457.

PÄTAU, K., 1935 Chromosomenmorphologie bei *Drosophila melanogaster* und *Drosophila simulans* und ihre genetische Bedeutung. Naturwissenschaften. **23:** 537–543.

PATTERSON, J. T., W. S. STONE, and A. B. GRIFFEN, 1940 Studies in the genetics of Drosophila XI. Evolution of the *virilis* group in Drosophila. Univ. Texas Pub. No. **4032:** 218–250.

RHOADES, M. M., 1940 Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics **25:** 483–520.

SLIZYNSKA, H., and B. M. SLIZYNSKI, 1941 A salivary gland chromosome map of *Drosophila funebris* Fabr. Proc. Seventh Int. Genetical Cong. 226.

SPENCER, W. P., 1927 The X chromosome of *Drosophila hydei*. J. Exp. Zool. **47:** 441–466.

1940 Subspecies, hydrids and speciation in *Drosophila hydei* and *Drosophila virilis*. Amer. Nat. **74:** 157–180.

STALKER, H. D., 1940 Chromosome homologies in two subspecies of *Drosophila virilis*. Proc. Nat. Acad. Sci. **26:** 575–578.

STUBBE, A., and M. VOGT, 1940 Über die Homologie einiger Augenfarbgene bei *Drosophila funebris* und *D. melanogaster* an Hand von Augentransplantationen. Z. i. A. V. **78:** 251–254.

STURTEVANT, A. H., 1918 A parallel mutation in *Drosophila funebris*. Science **48:** 72–73.

1921a Genetic studies on *Drosophila simulans*. II. Sex-linked group of genes. Genetics **6:** 43–64.

1921b Genetic studies on *Drosophila simulans*. III. Autosomal genes. General discussion. Genetics **6:** 179–207.

1921c A case of rearrangement of genes in Drosophila. Proc. Nat. Acad. Sci. **7:** 235–237.

1929 The genetics of *Drosophila simulans*. Carnegie Inst. Washington Publ. **399:** 1–62.

1932 The use of mosaics in the study of the developmental effects of genes. Proc. Sixth Int. Cong. Genetics **1:** 304–307.

1938a The homologies of the chromosome arms of the different species of Drosophila. Genetics **23:** 173–174.

1938b Essays on evolution III. On the origin of interspecific sterility. Quart. Rev. Biol. **13:** 333–335.

1939 On the subdivision of the genus Drosophila. Proc. Nat. Acad. Sci. **25:** 137–141.

1940 Genetic data on *Drosophila affinis*, with a discussion of the relationships in the subgenus Sophophora. Genetics **25:** 337–353.

STURTEVANT, A. H., and T. DOBZHANSKY, 1936 Observations on the species related to *Drosophila affinis*, with descriptions of seven new forms. Amer. Nat. **70:** 574–584.

STURTEVANT, A. H., and C. C. TAN, 1937 The comparative genetics of *Drosophila pseudoobscura* and *D. melanogaster*. J. Genet. **34:** 415–432.

TAN, C. C., 1935 Identification of the salivary gland chromosomes in *Drosophila pseudoobscura*. Proc. Nat. Acad. Sci. **21:** 200–202.

1937 The cytological maps of the autosomes in *Drosophila pseudoobscura*. Z. Zellf. Mik. Anat. **26:** 439–462.

TIMOFÉEFF-RESSOVSKY, N. W., 1927 Studies on the phenotypic manifestation of hereditary factors. I. On the phenotypic manifestation of the genovariation radius incompletus in *Drosophila funebris*. Genetics **12:** 128–198.

WEINSTEIN, A., 1920 Homologous genes and linear linkage in *Drosophila virilis*. Proc. Nat. Acad. Sci. **6:** 623–639.

WHITE, M. J. D., 1940 A translocation in a wild population of grasshoppers. J. Hered. **31:** 137–140.

WRIGHT, S., 1940 Breeding structure of populations in relation to speciation. Amer. Nat. **74:** 232–248.

THE ASSOCIATION OF MUTANTS WITH HOMOZYGOUS DEFICIENCIES IN ZEA MAYS

BARBARA McCLINTOCK
University of Missouri, Columbia, Missouri

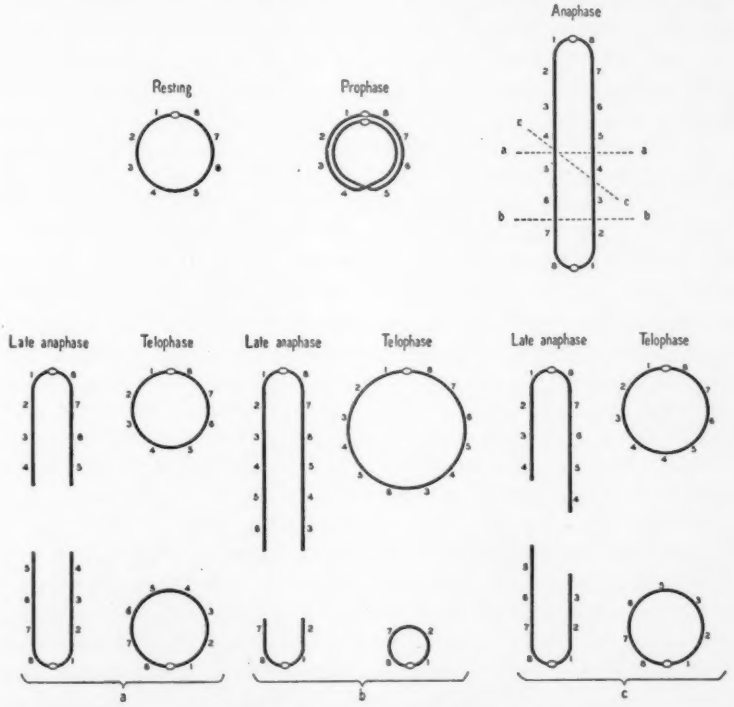
Received May 3, 1941

INTRODUCTION

THE evidence to be presented in this paper supports the supposition that some recessive mutants in maize are caused by homozygous minute deficiencies. A method by which homozygous minute deficiencies may be produced in maize has been presented in a previous publication (McCLINTOCK 1938). It is related to the aberrant mitotic behavior of ring-shaped chromosomes. It has been shown both in maize and Nicotiana (STINO 1940; R. E. CLAUSEN unpublished) that a chromosome in the form of a ring does not maintain itself unaltered through successive nuclear cycles. The details of this behavior have been presented in the two mentioned papers. They may be summarized briefly as follows: (1) Ring-shaped chromosomes may decrease in size by loss of segments of chromatin from the ring. (2) Ring chromosomes may increase in size by duplications of segments of chromatin composing the ring. (3) Ring chromosomes may be completely eliminated from sister telophase nuclei during a mitotic cycle. (4) The frequency of these occurrences depends upon the length of the chromonema composing the ring—the larger the ring chromosome, the more frequent the aberrant mitotic behavior. In maize, aberrant mitotic configurations producing alterations or elimination of the ring chromosomes may occur in 17 to 20 percent of all the division figures if the chromonema composing the ring is as long as that of the longest chromosome of the complement. If the ring chromosome is small—for example, composed of only four chromomeres—the aberrant mitotic configurations are very infrequent and may occur only once in every five or six hundred divisions. In all other nuclear divisions the ring chromosome behavior is normal; the two sister halves of the ring chromosome separate freely and pass to opposite poles of the spindle figure along with the rod chromosomes of the complement.

When aberrant configurations are produced by large ring-shaped chromosomes, the chromatin content of the ring chromosome usually is altered in each resulting sister telophase nucleus. Only rarely is the ring chromosome lost to one or both sister telophase nuclei. In contrast, aberrant mitotic configurations produced by small ring-shaped chromosomes usually result in total loss of the ring-shaped chromosome from both sister telophase nuclei. Only rarely do the telophase nuclei receive altered ring-shaped chromosomes.

The method by which ring-shaped chromosomes are lost to nuclei or become altered has been described previously (McCLINTOCK 1938). It may be outlined briefly as follows. At some mitotic prophases the two sister halves of a divided ring chromosome form a continuous double-sized ring chromosome instead of two freely separating ring chromosomes (Prophase, fig. 1). It is not known whether this results from the method of reduplication of the chromonema composing the ring chromosome or from a somatic crossover between the two sister chromatids subsequent to this reduplica-



DESCRIPTIONS OF TEXT FIGURES

FIGURE 1.—Diagram illustrating a method by which a ring chromosome becomes altered in chromatin constitution. Upper left: a ring chromosome in a resting nucleus. The clear oval represents the centromere. The individual parts of the ring chromosome are designated by the numerals. Upper middle: A prophase configuration following a "crossover" between the two sister chromatids of the divided ring chromosome. A dicentric, double-sized ring chromosome is produced. Upper right: Appearance of the dicentric ring chromosome in the following anaphase. Breakage of the chromatin strands between the centromeres may occur at any position. Three possible positions, a, b, and c, respectively, are indicated by the dash lines. The resulting broken strands at late anaphase and the new ring chromosomes formed at telophase by fusions of broken ends of these strands are diagrammed below in the bracketed figures for the breaks a, b, and c, respectively.

tion. At early anaphase, the two centromeres which are present in this double-sized ring chromosome move toward opposite poles of the spindle figure (Anaphase, fig. 1). The subsequent behavior in the spindle figure depends on the size of the ring chromosome. The ring chromosomes used in the investigations to be described in this paper were small. Therefore, the following description will be confined to their behavior. With such small double-sized ring chromosomes, continued movement of the centromeres is usually suspended and the double-sized ring chromosome remains in the region of the equatorial plate during anaphase. It is consequently eliminated from both sister telophase nuclei. Very occasionally, however, the strands of chromatin between the two centromeres become broken, and a segment of the double-sized ring chromosome enters each telophase nucleus. Fusion occurs between the broken ends of the segment. Consequently, a newly organized ring-shaped chromosome is produced in each of these telophase nuclei. Illustrations of several types of ring chromosomes which may be produced following breakage of a double-sized, two-centromere ring chromosome are given in figure 1. It will be seen that ring chromosomes with duplicated segments or ring chromosomes with deficient segments may be produced by this process. In either case, the size of the segment may be minute or relatively large. Although aberrant mitotic configurations, which could result in loss or change in size of the ring-shaped chromosomes, are relatively infrequent, it has been found that the frequency of loss of the ring-shaped chromosome from both telophase nuclei is considerably greater than the frequency of breakage of the ring chromosome with inclusion of broken segments in each of the sister telophase nuclei. This relationship is of considerable importance in the study to be described.

A simple method of obtaining sectors of a plant or even whole plants which are homozygous deficient for a small segment of a chromosome may be outlined as follows. It is necessary to have a rod-shaped chromosome from which a relatively short segment has been deleted and a ring-shaped chromosome composed of the chromatin deleted from the rod-shaped chromosome. The deficient rod chromosome plus the compensating ring-shaped chromosome are thus equivalent in chromatin content to a single normal rod chromosome. (See Df-1 and R-1; Df-2 and R-2, fig. 2.) Union of two gametes, each with a complete genomic complement but possessing the deficient rod chromosome and its compensating ring chromosome (instead of the normal rod chromosome), could give rise to plants with two homologous deficient rod chromosomes and two homologous compensating ring chromosomes. However, because of their small size, the ring chromosomes would frequently be lost to telophase nuclei following the formation of aberrant mitotic configurations. Thus, in the development of such plants, some nuclei would contain only a single ring chromosome following

loss of one of the ring chromosomes. During subsequent multiplication of such cells, an aberrant configuration could eliminate the second ring chromosome. Thus, cells would be produced whose nuclei have no chromatin segment covering the deficiency in the rod chromsomes. These cells would be homozygous deficient for the full extent of the deficiency in the rod chromosomes. (For this preliminary presentation, it may be assumed that these cells are inviable or incapable of further multiplication.) Very occasionally, however, an aberrant mitotic configuration of a ring chromosome would result not in loss but in a changed composition of the ring chromosome as illustrated in figure 1. If, during development of the sporophytic tissues, one of the ring chromosomes becomes reduced in size by loss of a minute segment and subsequently, the second ring chromosome is lost to a cell at a mitotic anaphase, all the cells arising from this latter cell could be homozygous deficient for a minute segment—the segment which had previously been deleted from the remaining ring chromosome. When the loss of a minute amount of genic substance results in a change in the genotype which is neither cell lethal nor interferes greatly with the capacity of such cells to multiply, a sector of tissue could be formed with a visibly modified phenotype. Minute deletions of different segments of the ring chromosome could be produced following such aberrant mitoses. In one plant an aberrant mitosis might result in the deletion of one particular segment from the ring chromosome. In another plant, an aberrant mitosis might result in the deletion of an entirely different segment. Thus, tissues homozygous deficient for different segments within the limits of the deficiency in the rod-chromosomes could be produced. If, when homozygous, each minute deficiency results in a particular type of visible modification of the tissue, a number of different and distinguishable types of mutant sectorials should be produced in these plants, each of which should be associated with loss of a particular segment from the ring. Furthermore, sectors showing identical characters should arise independently in a number of different plants if sufficient numbers of plants are available for observation. This is because a double-sized dicentric ring chromosome (fig. 1) could be broken at the same position on a number of independent occasions resulting in the production of altered ring chromosomes from which the same segment has been deleted.

If two adjacent segments, each of which when homozygous deficient produces a mutant character, are simultaneously deleted from the ring chromosome during an aberrant mitosis, a compound mutant sectorial should be formed showing the characters caused by each deficiency—that is, comparable to a condition of homozygosity for two recessive mutants. If a mutant sector in a plant is included in the inflorescence, gametes could be formed containing the deficient rod chromosome plus the deficient ring

chromosome. Fusion of two such gametes could give rise to plants which are homozygous for the minute deficiency and thus homozygous for the phenotypic expression which it induces. In such a way, altered ring chromosomes may be isolated.

TABLE 1

The appearance of the tissues of plants with two deficient rod chromosomes and two compensating ring chromosomes, Ring 1 and Ring 2, columns one and two, respectively. N represents a ring chromosome whose chromatin completely covers the deficiency in the rod chromosomes. a, b, and c represent ring chromosomes with minute deficiencies; each deficiency, when homozygous, gives rise to the phenotypic character a, b, and c, respectively. a b and b c represent ring chromosomes with two such minute deficiencies which, when homozygous, give rise to the compound mutant a b and b c, respectively. The appearance of the tissues when both rings are present is shown in column 3. The + indicates normal, non-mutant tissues. In columns 4 and 5 the phenotypic appearance of the tissues following somatic loss of Ring 1 and Ring 2, respectively, is indicated.

RING 1	RING 2	APPEARANCE OF TISSUES WHEN BOTH RINGS ARE PRESENT	APPEARANCE OF TISSUES FOLLOWING LOSS OF RING 1	APPEARANCE OF TISSUES FOLLOWING LOSS OF RING 2
N	N	+	+	+
N	a	+	a	+
N	b	+	b	+
N	c	+	c	+
N	a b	+	a b	+
N	b c	+	b c	+
a	b	+	b	a
a	c	+	c	a
a	a b	a	a b	a
a	b c	+	b c	a
b	c	+	c	b
b	a b	b	a b	b
b	b c	b	b c	b
c	a c	c	a c	c
c	b c	c	b c	c
a b	b c	b	b c	a b

The meaning of simple and compound mutants produced by altered ring chromosomes is important for an understanding of the logic of this paper. Therefore, a table has been prepared (table 1) to illustrate this meaning. Assume three adjacent segments in the ring chromosome each of which when homozygous deficient results in the characters **a, b,** and **c,** respectively. If, in individual plants, altered ring chromosomes are isolated with the simple deficiency mutants **a, b,** and **c,** respectively, and the compound mutants **a b** and **b c**, respectively, combinations of each of these altered rings with a normal ring chromosome or with any other altered ring chromosome may be made and should give predictable results. These are shown in the table along with the characters of the tissues which would be formed following mitotic loss of one or the other of the ring chromosomes, respectively.

It is the purpose of this paper to describe the types of simple and compound mutants which arise in plants possessing two homologous deficient rod-shaped chromosomes and one or more compensating ring-shaped chromosomes and to summarize the evidence which indicates that they are caused by homozygous minute deficiencies.

TWO CASES OF DEFICIENT ROD CHROMOSOMES PLUS COMPENSATING RING CHROMOSOMES

Two cases of deficient rod chromosomes with compensating ring chromosomes were available for this study. (For a complete description of their origin and behavior, see McCLINTOCK 1938). In both cases, segments of the short arm of chromosome 5 adjacent to the centromere were involved. The two cases are illustrated in figure 2. In the diagram, the numbers 1 to 9 in the normal chromosome 5 (first line in diagram) represent the positions of

FIGURE 2.—The upper figure is a diagram of a normal chromosome 5. The clear oval represents the centromere. The region adjacent to the centromere in the short arm is designated by the numerals 1 to 9. The middle figure represents a rod-shaped chromosome 5 deficient for the segments 1 to 4 (the Df-1 rod-chromosome). The dotted line represents the extent of the deficiency. To the right of this deficient rod chromosome, a ring chromosome is diagrammed possessing the segment which has been deleted from the rod chromosome (R-1). In a similar manner, the Df-2 rod chromosome and its compensating ring chromosome have been diagrammed.

the first nine chromomeres adjacent to the centromere. The rod chromosome, Df-1, is deficient for the four chromomeres adjacent to the centromere. Its compensating ring chromosome (R-1) possesses these four chromomeres (four chromomere ring below arrow, fig. E, Plate 1). The Df-2 rod chromosome (fig. 2) is deficient for these same four chromomeres plus the next five chromomeres. The R-2 ring chromosome possesses these nine chromomeres (fig. A, Plate 1; lower arrow, fig. B and G 1, Plate 1). It should be noted that the R-1 ring chromosome does not cover the deficiency in the Df-2 chromosome but the R-2 ring chromosome does cover the deficiency in the Df-1 chromosome. The transmission through gametes of the two deficient rod chromosomes without compensating ring chromosomes or with one or more ring chromosomes is given in table 2.

The types of plants most used in this investigation and the types of tissues which result from loss of the ring chromosome during development in these plants are summarized in table 3. In all cases, the tissues are normal when a single ring chromosome is present (either R-1 or R-2) covering the deficiency in the rod chromosomes. Tissues homozygous deficient for

TABLE 2

Transmissions through gametes of the deficient rod chromosomes without ring chromosomes or with various ring chromosomes. + represents transmission; − represents non-transmission.

	THROUGH ♀	THROUGH ♂
Df-1	+	−
Df-1 plus R-1	+	+*
Df-1 plus R-2	+	+*
Df-1 plus R-1 plus R-2	+	+*
Df-2	−	−
Df-2 plus R-1	−	−
Df-2 plus R-2	+	+
Df-2 plus R-1 plus R-2	+	+

* In a previous publication (McCLINTOCK 1938) it was shown that Df-1 was not transmitted through the pollen even when the deficiency was covered by a ring chromosome. Since this publication, a strain was obtained which will transmit the Df-1 chromosome through the pollen when a covering ring chromosome is present.

chromomeres 1 to 9 are completely inviable; tissues homozygous deficient for chromomeres 5 to 9 are likewise inviable. In contrast, the tissues which are homozygous deficient for chromomeres 1 to 4 are viable, but the growth capacity of these cells is very poor. Only very minute sectors of such homozygous deficient tissues are found. (See McCLINTOCK 1938, text fig. 31–38 and this paper, a, fig. 3.) The cells in these sectors are small. The lignified walls of cells with a full genomic complement are white, but the walls of cells homozygous deficient for chromomeres 1 to 4 are brown in color. There are no well developed plastids in these cells; therefore, the tissues of the leaf and stalk which are homozygous deficient for chromomeres 1 to 4 are not green and contrast strikingly with non-deficient tissues which are green. On exposure to direct sunlight, these cells soon die, and the

FIGURE 3.—a. Photograph of a part of a leaf of a Df-1/Df-2 plant with ring chromosomes R-1 and R-2. A sector showing the character of the blotch mutant is evident slightly to the right of the letter a. The wide band to the left of this letter is the mid-rib. The continuous parallel fine lines are the veins of the leaf. The shorter, narrower lines between these veins are the sectors of tissue homozygous deficient for regions 1 to 4 of chromosome 5 which arise following somatic loss of both the R-1 and the R-2 ring chromosomes. b. Photograph of part of a leaf of a Df-2/Df-2 plant with a two ring chromosomes, a normal R-2 ring and the brown-blotch-dries *I* ring. Two sectors showing the blotch character are visible to the right and left of the mid-rib, respectively. The photograph was taken before the tissue had commenced to disintegrate and dry. c. Similar to b. A wide sector showing the mutant character blotch-dries is present at each edge of the leaf. The disintegration and drying process has commenced at the outer edge of each sector. d. Leaves from plants of the constitution Df-1/Df-2 plus one R-2 ring chromosome. Note the solid (non-variegated) sector of mutant tissue (pink) in each leaf.

FIGURE 4.—Appearance of a young Df-2/Df-2 plant with two R-2 ring chromosomes, a normal R-2 ring and the brown-pink *I* ring. The plant is variegated for the compound mutant character brown and pink (white sectors in the photograph). These sectors arise following somatic loss of the normal R-2 ring.

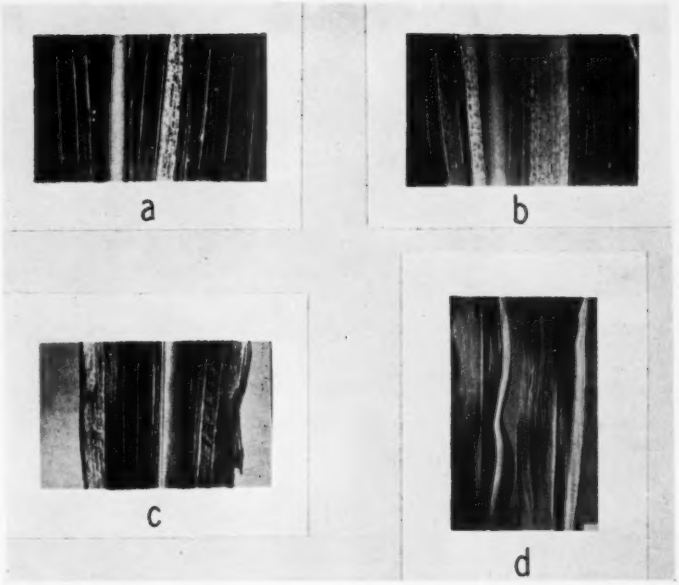


FIG. 3

FIG. 4

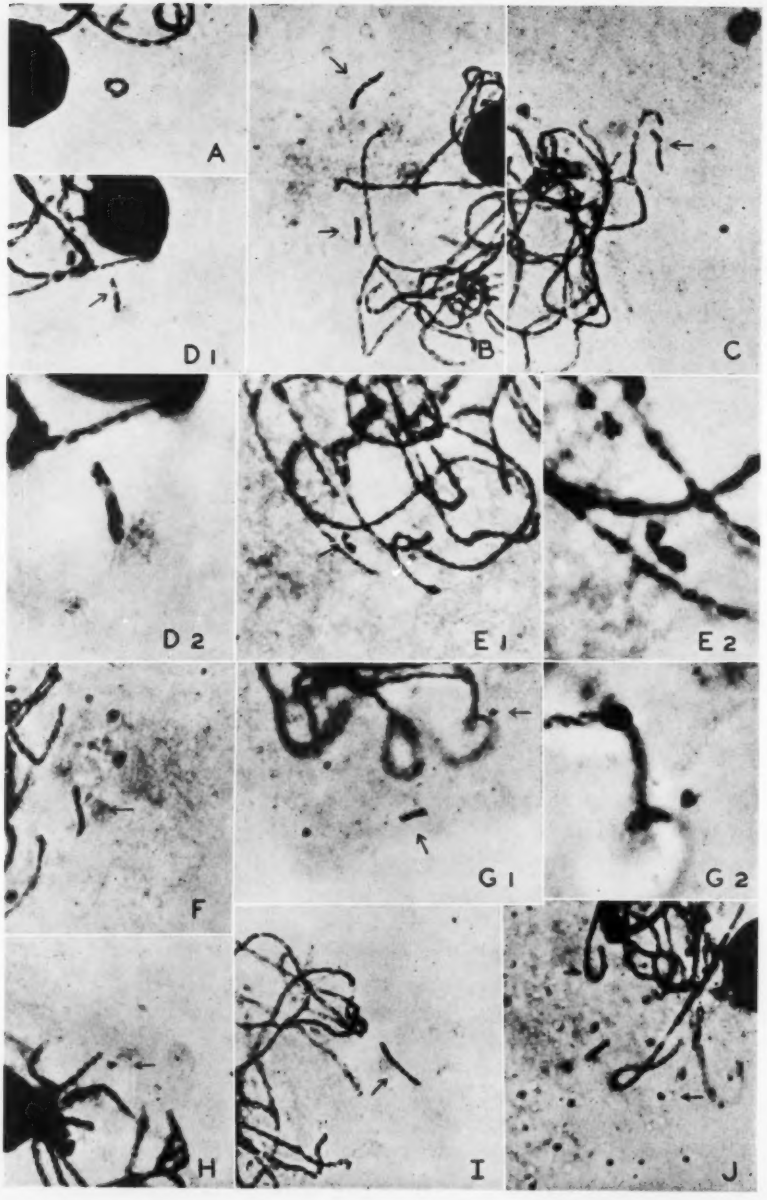

A
D1
B
C
D2
E1
E2
F
G1
G2
H
I
J

TABLE 3

Viability of tissues following somatic loss of ring chromosomes.
+, normal. −, very poor growth. o, inviable.

CHROMOSOME CONSTITUTION OF PLANTS		APPEARANCE OF TISSUES WITH COMPLETE COMPLEMENT	APPEARANCE OF TISSUES FOLLOWING SOMATIC LOSS OF RING CHROMOSOMES INDICATED IN THE COLUMNS							
DEFICIENT ROD-CHROMOSOME	RING CHROMOSOMES		1 R-1	2 R-1	1 R-2	2 R-2	1 R-1 plus 1 R-2	1 R-1 plus 2 R-2
Df-1/Df-1	1 R-1	+	−					
Df-1/Df-1	2 R-1	+	+	−				
Df-1/Df-2	1 R-2	+			−			
Df-1/Df-2	1 R-1 plus 1 R-2	+	+		+		−	
Df-1/Df-2	2 R-2	+			+	−		
Df-2/Df-2	2 R-2	+			+	o		
Df-2/Df-2	1 R-1 plus 2 R-2	+	+		+	o	+	o

DESCRIPTION OF FIGURES FOR THE PLATE

All photographs are of pachytene configurations in microsporocytes. All magnifications are approximately 1100× except D 2, E 2, and G 2, which are approximately 2750×.

FIGURE A.—Synaptic association of two R-2 ring chromosomes.

FIGURE B.—The lower arrow points to a collapsed normal R-2 ring. The upper arrow points to a collapsed brown-pink *II* ring chromosome which is approximately double the size of the normal R-2 ring.

FIGURE C.—Two collapsed ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal R-2 ring is below the arrow. Above is the reduced ring chromosome which produces the brown *I* phenotype.

FIGURE D 1.—Two collapsed ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal four chromomere R-1 ring (above) is slightly out of focus. The ring below the arrow is a reduced R-2 ring composed of seven chromomeres. It gives rise to the brown-pink *IV* phenotype. D 2. Enlargement of the same.

FIGURE E 1.—Two ring chromosomes associated at their centromeres. The arrow points to the associated centromeres. The normal, four chromomere R-1 ring lies below the arrow. An R-2 ring reduced to three chromomeres lies above the arrow. This latter ring gives rise to the brown-blotchdries *II* phenotype. E 2. Enlargement of the same.

FIGURES F and H.—The arrows point to the collapsed ring chromosomes in two sister cells of a Df-1/Df-2 plant with one R-2 ring chromosome. All surrounding cells possessed a normal R-2 ring. In one of these sister cells, fig. F, the R-2 ring chromosome was enlarged. In the other cell, fig. H, the R-2 ring chromosome was reduced to two chromomeres. In chromatin constitution the two rings are equivalent to two normal R-2 ring chromosomes.

FIGURE G 1.—A collapsed normal R-2 ring chromosome (lower arrow) and an R-2 ring chromosome reduced to two chromomeres (upper arrow). This latter ring chromosome gives rise to the compound mutant brown-light green-poor growth. G 2. Enlargement of the same showing this latter reduced ring chromosome.

FIGURE I.—The arrow points to the collapsed double-sized R-2 ring chromosome which gives rise to the brown-pink *II* phenotype.

FIGURE J.—Pachytene configuration in a plant with three ring chromosome. A collapsed normal R-2 ring lies to the left. A normal R-1 ring lies immediately above it. The arrow points to the reduced R-2 ring chromosome which produces the brown-light green-poor growth phenotype.

tissue disintegrates and dries. Because cells and tissues completely deficient for these four chromomeres not only are viable but also show phenotypic modifications, it is expected that tissues deficient for segments within this region would not only be viable, but could have much better growth rates and might show specific characters.

Studies of somatic mitosis have shown that the larger of the two ring chromosomes (R-2) produces a double-sized, dicentric ring chromosome (Prophase, fig. 1) approximately once in every hundred nuclear divisions. All of the other nuclear divisions are completely normal; the ring chromosome divides normally, the two free sister halves pass to opposite poles along with the rod chromosomes. In nearly all cases, when a double-sized ring chromosome is formed at prophase, it remains in the region of the equatorial plate during anaphase and telophase and is thus completely eliminated from the sister telophase nuclei. Very occasionally, however, a double-sized dicentric R-2 ring chromosome becomes broken at anaphase, and a segment of the broken double-sized ring chromosome enters each sister telophase nucleus. In both telophase nuclei fusion of broken ends occurs, reestablishing a ring-shaped chromosome, but the content of the ring-shaped chromosome is usually altered. Similar studies of the smaller ring chromosome (R-1) have shown that aberrant anaphase behavior occurs approximately once in every 600 nuclear divisions. In this case, due to its small size, the ring chromosome is nearly always lost to the sister telophase nuclei. Only rarely have altered R-1 rings been detected. The alterations in the chromatin content of the ring chromosomes to be described in this paper involve the R-2 ring.

DETECTION OF ALTERED RING-SHAPED CHROMOSOMES BY MEANS OF MUTANT SECTORS IN THE DF-1/DF-1, DF-1/DF-2 AND DF-2/DF-2 PLANTS

As stated in the previous section, alterations of the R-2 ring are considerably more frequent than alterations of the R-1 ring. Consequently, plants with one or two R-2 rings have shown the greatest number of detectable alterations. The plants most suitable for this study have had the following constitutions: Df-1/D-2 plus one R-2 ring; Df-1/Df-2 plus two R-2 rings; Df-2/Df-2 plus two R-2 rings.

In plants of the constitution Df-1/Df-2 plus one R-2 ring, a solid sector of modified tissue should appear following an alteration in the ring chromosome which produces a visible change in the character of the tissues. The size of the sector would depend upon when the altered ring chromosome arises in the development of the plant and the subsequent rate of growth of the cells possessing this ring. If the alteration occurred in a single nuclear division early in the development of the plant, a wide mutant sector could

result. If it occurred in a nuclear division late in development, only a small mutant sector would be produced. In Df-1/Df-2 plants with two ring chromosomes (either one R-1 plus one R-2 or two R-2) or in Df-2/Df-2 plus two R-2 rings, both solid sectors and sectors which are variegated for the mutant characters should be produced. The solid sectors should arise in the tissues of the plant which have only a single ring chromosome—that is, subsequent to a mitotic loss of one of the ring chromosomes. An alteration in the remaining ring chromosome could give a solid sector with a modified appearance. Variegated mutant sectors should arise when two rings are initially present in the tissue, one of which has become altered in an earlier mitotic division. When the two rings are present, a deficiency in the altered ring chromosome would be covered by the presence of this segment in the normal ring chromosome. No character caused by the deficiency in the altered ring chromosome could appear until after the normal ring chromosome has become lost in a later nuclear division. All the cells which arise from a cell which has lost the normal ring chromosome would be able to show the mutant character associated with the deficiency in the altered ring chromosome. Since loss of the normal ring chromosome should occur independently in a number of cells, a sector which is variegated for the mutant character should be produced. The appearance of a solid sector in a plant with a single ring chromosome is illustrated in a and d of figure 3; the appearance of variegated sectors in two-ring plants is illustrated in b and c of figure 3 and in figure 4.

If a particular mutant character is associated with loss of a particular region from the ring chromosome—that is, if the character is produced by a homozygous minute deficiency—only a restricted number of mutant characters should appear in the Df-1/Df-1 and Df-1/Df-2 plants. The segment within which homozygous deficiencies may be detected is only four chromomeres long (fig. 2). If this theory is correct, the same mutant characters which appear in the Df-1/Df-1 and Df-1/Df-2 plants should likewise appear in the Df-2/Df-2 plants because homozygous deficiencies within regions 1 to 4 (chromomeres 1 to 4, fig. 2) may be produced in all three types of plants. However, other mutants which are not found in the Df-1/Df-1 and Df-1/Df-2 plants should be present in the Df-2/Df-2 plants. These characters should be related to homozygous deficiencies within the region 5 to 9 (chromomeres 5 to 9, fig. 2). Such mutant characters should not appear in the Df-1/Df-1 or Df-1/Df-2 plants because the Df-1 chromosome possesses chromomeres 5 to 9, and thus no homozygous deficiencies can be produced within this region following aberrations of the ring chromosome. These anticipations have been completely fulfilled. Since the altered ring chromosomes may be isolated following the formation of gametes with a deficient rod chromosome and an altered ring chromosome,

it has been possible to conduct tests which show that a certain group of characters is associated with region 1 to 4 and another group of characters with region 5 to 9.

The frequency of mutant sectors is greater in the Df-1/Df-2 plants with one or two R-2 rings than in the Df-2/Df-2 plus two R-2 rings. The evidence, which is conclusive but which cannot be considered here because of limitations in space, indicates that this is due to changes in the R-2 ring chromosomes which have deleted rather large segments from the ring. In a Df-2/Df-2 plant, the cells with such large homozygous deficiencies are either inviable or produce tissues with such poor growth rates that they are not suitable for precise character studies. However, removal of a large segment from the R-2 ring in a Df-1/Df-2 plant may result in quite viable tissues with readily identifiable mutant characters. As an example, removal of region 4 to 8 in the R-2 ring in a Df-2/Df-2 plant would result in cells homozygous deficient for this region. These cells are inviable. In a Df-1/Df-2 plant, only region 4 could be homozygous deficient because the segment 5 to 8 covering the deficiency in the ring chromosome is present in the Df-1 rod chromosome.

Considering the number of mitoses taking place in the development of a plant, the occurrence of an abnormal mitosis which results in an altered ring chromosome is extremely rare. Once an altered ring chromosome has been isolated, it can be maintained unchanged through as many plant generations as desired by avoiding those plants in which this ring chromosome has again become altered. There are relatively few such plants.

MUTANT CHARACTERS ASSOCIATED WITH REGION 1 TO 4 OF CHROMOSOME 5

As stated previously, tissues which are homozygous deficient for the segments 1 to 4 of chromosome 5—the full extent of the deficiency in the Df-1 rod chromosome—are capable of multiplication but at a very slow rate. Consequently, only minute visible sectors are produced following complete loss of the ring chromosomes in Df-1/Df-1 or Df-1/Df-2 plants. The mutant characters shown by these homozygous deficient tissues are: brown cell walls, colorless plastids, very poor growth capacity, and disintegration and drying of the tissue when exposed to direct sunlight. It is to be expected that deficiencies for minute segments within this region should be viable. They may possess very much better growth capacities and could show mutant characters. Thus, relatively wide sectors showing mutant characters associated with a minute homozygous deficiency within region 1 to 4 might be produced in these plants. In the Df-1/Df-1 or Df-1/Df-2 plants, all the mutant characters observed as sectorials should be related to changes within region 1 to 4 of chromosome 5. In the Df-2/Df-2 plants, these same types of mutant characters should appear

plus additional mutant characters which are not found in the former types of plants. These latter mutants should be related to changes within region 5 to 9.

The various types of mutant characters having a clearly recognizable phenotypic expression which have appeared as sectorials in all three types of plants—that is, those mutants associated with region 1 to 4 of chromosome 5—have been divided into three groups: (1) Those which show a single recognizable character in the mutant sector (simple mutants); the tissue may have a normal growth rate. (2) Those which exhibit two or more of these characters in a single sector (compound mutants); the tissues may have a normal growth rate. (3) Sectors with distinct mutant characters. These sectors are always narrow—that is, associated with a reduced growth rate of the cells of the sector; many of these are recognizable as compound mutants.

The simple mutants to be described involve some obvious color change. Although other mutants would be expected and are probably present, their positive detection as sectorials in these plants is both difficult and uncertain. Therefore no attempt was made to isolate mutants which did not produce an unmistakable character change which could express itself as a sector in a plant. The color mutants belong to the unmistakable class.

Group 1. Simple mutants which may have a normal growth rate

Brown cell walls

The lignified cell walls are brown in color. This character is similar in all details to the character produced by the recessive mutant brown mid-rib (symbol, *bm*) previously located within the region 1 to 4 of a normal rod chromosome 5. Henceforth the character will be designated as brown. The normal R-1 and R-2 rings are known to carry the dominant allele of *bm* (*Bm* colorless cell walls). (For a complete description of the *bm* phenotype and proof of the presence of *bm* in the normal ring chromosomes, see McClintock 1938.) Three different ring chromosomes producing the brown phenotype have been isolated. Two appear to be normal in size and could not be distinguished from normal R-2 rings. Either ring will produce the brown character in Df-1/Df-1, Df-1/Df-2 or Df-2/Df-2 plants. The third brown ring is reduced to six chromomeres (upper collapsed ring, fig. c, Plate 1). When this ring is present, brown will appear only in Df-1/Df-1 or Df-1/Df-2 plants. In Df-2/Df-2 plants the cells which have only this ring are inviable. In this case, the three chromomere deficiency in the ring chromosome probably includes a segment of region 5 to 9 which could express itself as a homozygous deficiency only in the Df-2/Df-2 plants. As explained previously, in the Df-1/Df-1 or Df-1/Df-2 plants, the cells with only this reduced ring chromosome might possess only a minute homo-

zygous deficiency and thus be quite viable. This would explain the difference in viability of the cells possessing only this ring in the three types of plants.

Pink

Chlorophyll is absent although plastids are present. In the stalk, the number of layers of cells from the outer circle of bundles to the epidermis frequently is greater in the pink sectors than in the non-modified parts of the plant. Consequently, the pink sector produces a protuberance. On the stalk and in the upper leaves of the growing plant, the color is an intense salmon pink. As the leaves mature, the pink color gradually fades to white. Two ring chromosomes giving rise to the pink phenotype have been isolated, but the constitution of only one of these rings has been investigated cytologically. It is an R-2 ring with no clearly observable change in chromatin constitution. It produces the pink phenotype in either a Df-1/Df-1, a Df-1/Df-2 or a Df-2/Df-2 plant.

Blotch

The chlorophyll pattern is finely speckled (a, fig. 3). No ring chromosome producing a blotch character associated with a good growth capacity has been isolated so far, although a large number of such sectorials have been observed in both Df-1/Df-2 and Df-2/Df-2 plants. Several ring chromosomes producing blotch but associated with poor growth capacities have been isolated. None of these has been investigated extensively.

Blotch-dries

This character is similar to blotch although the chlorophyll color usually is less intense. In the young leaf, the character is first detected as a light green sector. The blotch pattern of the chlorophyll is acquired as the leaf matures. After a short time, the cells in the sector which have been exposed to direct sunlight begin to disintegrate. Finally, the whole sector so exposed becomes a mass of dead, dried tissue (see b and c, fig. 3). Two ring chromosomes producing blotch-dries have been isolated recently. Neither has been sufficiently investigated as yet.

Group 2. Compound mutants which may have normal growth rates

Brown-pink

Many sectors showing the combined characters of brown and pink have been observed in all three types of plants. Four different ring chromosomes producing this compound character have been isolated. One is an obviously reduced R-2 ring chromosome composed of seven chromomeres (lower collapsed ring, fig. D, Plate 1). Two are slightly reduced R-2 rings, and one is an enlarged R-2 ring, approximately double the size of the normal R-2 ring (collapsed ring, upper arrow, fig. B and fig I, Plate 1). The

brown-pink character produced by the seven-chromomere ring appears only in the Df-1/Df-1 or Df-1/Df-2 plants. In the Df-2/Df-2 plants, the cells with only this ring chromosome are inviable. The other three ring chromosomes will produce the brown-pink character when present in either a Df-1/Df-1, a Df-1/Df-2, or in a Df-2/Df-2 plant.

Brown-pink-dries

At the initial stage, the sectors showing this compound mutant character are strictly comparable to the compound mutant brown-pink. However, as in blotch-dries, on exposure to sunlight, the cells die and the tissue dries. These sectors appear in both Df-1/Df-2 and Df-2/Df-2 plants. Several rings causing this compound mutant character have been isolated, but none has been investigated fully.

Brown-blotch-dries

The sectors exhibiting this compound mutant are identical in appearance and behavior to the blotch-dries sectors described above. In addition, however, the lignified cell walls are brown. Two ring chromosomes producing this compound mutant have been isolated. One is a slightly enlarged R-2 ring. The other is a much reduced ring chromosome composed of only three chromomeres (ring above arrow, fig. E, Plate 1). When the former ring chromosome is present, the character will appear in either a Df-1/Df-2 or a Df-2/Df-2 plant. When the latter ring is present, the compound mutant character will appear only in a Df-1/Df-1 or a Df-1/Df-2 plant. In the Df-2/Df-2 plants, the cells which possess only this ring chromosome are inviable—probably because of the six chromomere deficiency.

Group 3. Compound mutants whose growth rates are considerably reduced

Brown-pink-dries-poor growth

The sectors showing this group of characters are similar in appearance to the compound mutant brown-pink-dries of group 2. However, the growth rate of the mutant tissues is always considerably reduced. Thus, only narrow sectors are formed. Three rings producing this compound mutation have been isolated, but only one has been sufficiently studied. It is a slightly reduced R-2 ring. The character which this ring produces is exactly the same in the Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. Thus, the phenotypic modification which this ring chromosome induces is probably restricted to a segment within region 1 to 4 of chromosome 5.

Light green-poor growth

The chlorophyll color in these sectors is a light green, but the growth rate of the cells is so reduced that sectors are not wider than 3 or 4 mm.

Only one ring chromosome producing this character has been isolated. It possesses only two chromomeres. Thus, the character associated with this ring appears only in the Df-1/Df-1 and Df-1/Df-2 plants. It does not appear in the Df-2/Df-2 plants due to the extensive deficiency producing inviability in the cells possessing only this ring.

Brown-light green-poor growth

Sectors showing this character are similar in appearance to those described above. In addition, the lignified cell walls are brown. Only one ring chromosome giving rise to this compound mutant has been isolated. It possesses two chromomeres (upper arrow, fig. G 1 and enlargement, fig. G 2, Plate 1). Due to the extensive deficiency in this ring chromosome, the characters associated with this ring appear only in Df-1/Df-1 or Df-1/Df-2 plants.

Many sectors exhibiting very poor growth capacities associated with a blotched chlorophyll pattern or with colorless tissues, either with or without brown, have been observed in the Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. No attempt has been made to isolate the rings responsible for such characters because of the impracticability of work with characters having such poor growth rates.

MUTANT CHARACTERS ASSOCIATED WITH REGION 5 TO 9 OF CHROMOSOME 5

The mutant characters which may be associated with alterations in the R-2 chromosome within region 5 to 9 are those which do not appear in the Df-1/Df-1 or Df-1/Df-2 plants but appear only in the Df-2/Df-2 plants. It is difficult to obtain sufficient seed for an extensive investigation of the types of mutants appearing in this latter type of plant. Only one to several kernels are likely to occur on an ear of a Df-2/Df-2 plant, since the Df-2 chromosome is not transmitted through the female gametophyte without the compensating R-2 ring chromosome. During meiosis, the R-2 rings are so frequently eliminated that few megaspores are formed possessing this ring, and thus few seed may develop. Among the several thousands of plants which have been examined, a number of mutant sectorials have been observed. Many of these are related to changes in region 1 to 4 of the R-2 ring chromosome. Only a few clear-cut mutants have been observed which may be related to changes within region 5 to 9. In these plants, many mutant sectorials are composed of tissues which grow too poorly to be useful for character studies. No attempt has been made to isolate the changed rings responsible for the mutations with very poor growth rates. When a whole plant or half of a plant was variegated for such a character, a cytological study was made to determine the types of ring chromosomes which

were present. In all examined cases, one of the ring chromosomes was decidedly reduced. Following loss of the normal ring chromosome to some cells during development, a relatively large homozygous deficiency had to be present. If these cells reproduce at a very reduced rate, only very narrow sectors of homozygous deficient tissue could be formed. The presence of a reduced ring in all examined plants showing variegated sectors with considerably reduced growth rates leads one to suspect that the cells of these sectors possess only the reduced ring chromosomes—that is, they are homozygous deficient for the segment which had been deleted from the ring chromosome.

Of the mutants which possess a good growth capacity, only three have been found which may be ascribed to region 5 to 9; and up to the present time, only two of these have been proven to be in this region. These mutants are:

Pale green

The chlorophyll color in the leaves and the stalk is a pale green. The pale green character does not develop in the very young plants but becomes very striking in the older plants. The growth rate of this tissue is usually quite normal. Only one pale green ring has been isolated. No obvious change in the chromatin constitution of this R-2 ring was observed.

Striate

The sectors showing this character are composed of streaks of yellow-green tissues. Only one ring chromosome has been isolated which produces this character. This R-2 ring is slightly reduced. A gamete with the Df-2 rod chromosome and the R-2 striate ring chromosome is transmitted through the female gametophyte but not through the pollen. The location of the character within region 5 to 9 has been determined by use of a third deficiency rod chromosome (Df-3) possessing a small deficiency located within the limits of region 5 to 9. Striate appears when the striate ring chromosome is present in Df-2/Df-3 plants.

White

Sectors with a chalk white color have been observed in the Df-2/Df-2 plants but ring chromosomes producing white have not been isolated as yet. Since similar sectors have not been observed in many thousands of Df-1/Df-2 plants, it is assumed, although not proven, that this character is related to an alteration within region 5 to 9.

The pale green ring is the only one of this series which has been extensively investigated. It is not related to striate, since the pale green character does not appear in Df-2/Df-3 plants, whereas striate does appear in these plants. The following tests have shown that the pale green character

is related to region 5 to 9 and not to region 1 to 4 of chromosome 5. Following isolation of the pale green ring, plants were obtained with the constitution Df-1/Df-2 plus the pale green ring. These plants were indistinguishable from those of a similar constitution but having a normal R-2 ring. Thus, pale green does not appear in a Df-1 background. The functional male gametes produced by these plants possess the Df-2 rod chromosome plus the pale green ring chromosome. When pollen of such plants was placed on silks of plants of the constitution Df-2/Df-2 plus two normal R-2 ring chromosomes, all the resulting individuals (Df-2/Df-2 plus one normal R-2 ring plus the pale green R-2 ring) were variegated for pale green. The variegation arose as a consequence of the mitotic losses of the normal R-2 ring chromosome contributed by the female parent, thus giving rise to tissues containing only the pale green ring chromosome. It is in these tissues that the pale green character appears.

CYTOLOGICAL OBSERVATIONS OF THE RING-SHAPED CHROMOSOMES WHICH GIVE RISE TO THE MUTANT CHARACTERS

A pachytene configuration showing the association of two normal R-2 ring chromosomes is shown in figure A, Plate 1. When a single R-2 ring chromosome is present or when two R-2 ring chromosomes which have failed to associate at the meiotic prophase are present, the ring chromosome is collapsed (lower arrow, fig. B and G, Plate 1; ring below arrow, fig. C, Plate 1). This results from non-homologous associations of the chromatin forming the ring (McCLINTOCK 1933, 1938).

Following the formation of a double-sized dicentric ring chromosome in a mitotic prophase and its breakage in the following anaphase, an enlarged ring chromosome may be formed in one telophase nucleus and a reduced ring chromosome in the sister telophase nucleus (see fig. 1). This situation is illustrated by the constitution of the ring chromosome in two sister cells (arrows fig. F and H, Plate 1). These two sister cells are in the prophase of meiosis; therefore the event which resulted in the formation of these two altered ring chromosomes occurred at the last premeiotic anaphase. In individual cases, R-2 rings have been observed with various degrees of reduction in chromatin constitution. These range from a barely observable deficiency in the ring chromosome to deficiencies so large that the ring chromosome was composed of but a single large chromomere. Likewise, in individual cases, R-2 ring chromosomes have been observed with duplicated segments varying in extent from a very small duplication to one which produced a ring chromosome eight times the size of the normal R-2 ring.

Cytological observations of the composition of the ring chromosomes which produce visible mutations are summarized in table 4. As stated pre-

viously in the description of the mutant characters, several ring chromosomes have been isolated which produce the same mutant character (simple mutant) or the same group of mutant characters (compound mutant). Although the character which each of these ring chromosomes produces appears to be the same, the event which is responsible for the character which the ring chromosome produces occurred independently in each case in a single Df-1/Df-1, Df-1/Df-2, or Df-2/Df-2 plant. The numerals *I*, *II*, and *III*, etc., have been used to distinguish ring chromosomes producing the same character or the same group of characters which have originated independently of one another. For example, brown *I* was isolated from a sector of a Df-1/Df-2 plant, brown *II* from a sector of another Df-1/Df-2 plant, and brown *III* from a Df-2/Df-2 plant. Where a photographic illustration of a particular altered ring chromosome is reproduced in this paper, the figure reference is indicated in the table. Four of the fifteen ring chromosomes showed no observable change in the constitution of the R-2 ring. All four ring chromosomes are associated with simple mutants (brown *II*, brown *III*, pink, pale green). In nine cases, the ring chromosome was reduced. In two cases, the ring chromosome was enlarged (brown-pink *II*, brown-blotch-dries *I*). The brown-pink *II* ring, when first observed, was approximately double the size of the normal R-2 ring (upper arrow fig. B and fig. I, Plate 1). The brown-blotch-dries *I* ring is only slightly enlarged. In both of these cases, the alteration in the ring chromosome, which is responsible for the character the ring chromosome produces, could have occurred subsequent to a previous alteration which had enlarged the R-2 ring chromosome. It is likewise conceivable that the enlargement of the ring chromosome could have occurred following the alteration which produces the character but before isolation of the ring. It is known that the enlargement itself is not the factor responsible for the appearance of the character which such an enlarged ring chromosome produces. This is clearly indicated by the enlarged brown-pink *II* ring. This ring, when first isolated, was approximately double the size of a normal R-2 ring chromosome. Because of the increased length of the chromonema of this ring chromosome, aberrant mitoses involving this ring chromosome occur with increased frequency. Furthermore, the added length of the chromonema of this ring chromosome brings it into the range where aberrant mitoses may frequently lead to recoverable alterations in the ring chromosome. Because of its size, it was expected that this ring chromosome would not maintain itself but would tend to become reduced to the size where aberrant mitoses would lead most frequently to elimination of the ring chromosome rather than alterations in its chromatin content. This proved to be true. Several strains have been isolated in which this ring chromosome is only slightly larger than the normal R-2 ring chromosome. At this reduced size, the

ring chromosome tends to remain stable because aberrant mitoses now tend to eliminate the ring rather than alter its chromatin composition. It should be emphasized, however, that the characters produced by the brown-pink *II* ring following its reduction in size from a double-sized ring to one only slightly larger than a normal R-2 ring have not been altered by the reduction. It is assumed that even in its enlarged state, the brown-pink *II* ring chromosome possessed a minute deficiency which was responsible for the compound mutant character it produced. No duplications of the remaining segments of the ring chromosome or subsequent deletions of these duplicated segments should alter the expression of the character, for the minute deficiency would still be present regardless of the presence or absence of these duplicated segments.

TABLE 4

Chromatin constitution of the ring chromosomes which produce mutant characters.

1. No observed change in constitution of the R-2 ring.
   brown *II;* brown *III;* pink; pale green.
2. Slightly reduced R-2 ring: Loss of approximately one chromomere.
   brown-pink *I;* brown-pink *III;* striate.
3. Obviously reduced R-2 ring: Loss of two or three chromomeres.
   brown-pink *IV* (fig. D, Plate 1); brown-pink-dries-poor growth; brown *I* (fig. C, Plate 1).
4. Very reduced ring: Loss of six or seven chromomeres.
   brown-blotch-dries *II* (fig. E, Plate 1); light green-poor growth; brown-light green-poor growth (fig. G, Plate 1).
5. Enlarged ring: Larger than a normal R-2 ring.
   brown-pink *II*, approximately double the size of a normal R-2 ring (fig. B and I, Plate 1); brown-blotch-dries *I*, approximately ½ larger than the normal R-2 ring.

In 11 of the 15 cases it has been established that the ring chromosome has undergone an alteration. It would require considerable space to indicate the methods used which allow one to be certain that the ring chromosome being observed in a particular plant is the ring chromosome responsible for the character ascribed to it. The methods will be considered in a separate report.

TRANSMISSIONS OF ALTERED RING CHROMOSOMES THROUGH THE MALE AND FEMALE GAMETES

Tests have been made of the transmissions of the various altered ring chromosomes through the female gametophyte with the Df-1 or Df-2 rod chromosomes and through the pollen with the Df-2 rod chromosome. These results are summarized in table 5. Transmissions through the pollen of the various altered ring chromosomes with the Df-1 rod chromosome have not been included in the table, since a male-transmissible strain of Df-1 has only recently been obtained, and the tests have not been completed. The cytological determination of the type of ring chromosome associated with

each particular mutant has been included in column 2 of this table, since it allows the tests to be more readily interpreted. Since many of these altered ring chromosomes are readily transmitted through the pollen, even in competition with gametes carrying normal ring chromosomes, it has been possible to obtain various combinations of the ring chromosomes in Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants. These will be described in the following section.

TABLE 5

Gametic transmissions of the altered ring chromosomes with the Df-1 or Df-2 rod chromosomes. Transmissions of the altered ring chromosomes with the Df-1 rod chromosome through the pollen have not been included in the table because these tests have not been completed. + represents transmission. o represents no transmission. – indicates the test has not been completed.

RING CHROMOSOME	CHROMATIN CONSTITUTION OF ALTERED R-2 RING	TRANSMISSIONS THROUGH ♀ WITH:		TRANSMISSION THROUGH ♂ WITH
		Df-1	Df-2	Df-2
brown *I*	Reduced to six chromomeres	+	o	o
brown *II*	No apparent reduction	+	+	+
brown *III*	No apparent reduction	+	+	+
pink	No apparent reduction	+	+	+
pale green	No apparent reduction	+	+	+
striate	Slightly reduced	–	+	o
brown-pink *I*	Slightly reduced	+	+	+
brown-pink *II*	Enlarged	+	+	+
brown-pink *III*	Slightly reduced	+	+	+
brown-pink *IV*	Reduced to seven chromomeres	+	o	o
brown-pink-dries-poor growth	Slightly reduced	+	+	o
brown-blotch-dries *I*	Slightly enlarged	+	+	+
brown-blotch-dries *II*	Reduced to three chromomeres	+	o	o
light green-poor growth	Reduced to two chromomeres	+	o	o
brown-light green-poor growth	Reduced to two chromomeres	+	o	o

THE CHARACTERS PRODUCED IN PLANTS FOLLOWING COMBINATIONS OF ALTERED RING CHROMOSOMES: PROOF THAT THE COMPOUND MUTANTS ARE COMPOSED OF TWO OR MORE OF THE SIMPLE MUTANTS

It is obvious from the description given earlier in this paper that the characters exhibited by the compound mutants bear a relation to the simple mutants. The compound mutants appear to be composed of a combination of two or more of the observed simple mutants. It was also obvious in this study that the sectorials showing compound mutant characters occurred far more frequently than those showing a simple mutant character. Sectorials showing a simple mutant character were relatively rare. The method by which ring chromosomes become reduced in size at a mitotic anaphase would suggest that adjacent blocks of chromatin should fre-

quently be deleted from the ring chromosome following an aberrant mitosis which results in an alteration in the ring chromosome. If each of the simple mutants is produced following loss of a particular minute segment from the ring chromosome, then the compound mutants should be produced following loss of two or more of these segments. It would be expected that breakage of the double-sized ring at a mitotic anaphase would delete a block of chromatin more frequently than a very minute segment. Thus, compound mutants should appear far more frequently than simple mutants. With this interpretation, one should be able to deduce the order of the various segments in the ring chromosome which produce a particular character when homozygous deficient, merely by observing the characters which are present in the compound mutants. For illustrative purposes, one may use the characters pink, brown, and blotch dries. The most frequent types of compound mutations involving these characters are: brown-pink, brown-pink-dries, and brown-blotch-dries. Since brown may be with either pink or blotch dries, the suggested order is (1) pink followed by (2) brown followed by (3) blotch dries. Loss of the first two adjacent segments would give rise to the compound mutant brown-pink; loss of the second and third adjacent segments would give rise to the compound mutant brown-blotch dries; loss of all three adjacent segments would give rise to the compound mutant brown-pink-dries.

Proof that the compound mutants are composed of two or more of the simple mutants may be obtained readily. It is known that all of the mutant types behave as recessives. It is only necessary to combine two altered rings each of which gives a character or a group of characters in a Df-1/Df-2 or a Df-2/Df-2 plant and observe the characters of the tissues which possess the two altered ring chromosomes. The method is essentially that which is diagrammed in table 1, and the results are in complete agreement with it.

Various combinations of the altered ring chromosomes have been made. Those which involve the mutants brown, pink, brown-pink, brown-pink-dries, brown-blotch-dries are given in table 6. The appearance of the cells and tissues possessing both ring chromosomes are given in column 2. In column 3, the character of the tissues which possess only one or the other of the altered ring chromosomes (following mitotic loss of one or the other ring chromosome, respectively) has been indicated. Where more than one similar combination was made, the particular altered ring chromosome (that is, *I*, *II*, or *III*) is given below the character in each specific combination. It made no difference which ring chromosome producing a given mutant type was used. In every tested case the results were the same. Combination 1 of table 6 (X plus Normal) gives the characters resulting from the combination of any one altered ring chromosome, X, with a normal R-2 ring chromosome. In all of the 15 isolated cases the normal R-2 ring chro-

mosome suppressed the character produced by the altered ring chromosome when both were present in the same cell. However, the presence of the altered chromosome in these plants was made obvious by the variegation which appeared (fig. 4). This variegation resulted from the mitotic losses of the normal R-2 ring chromosome. The character produced by the altered ring chromosome could then be expressed.

TABLE 6

Phenotypic characters in Df-1/Df-1, Df-1/Df-2, and Df-2/Df-2 plants with various combinations of ring chromosomes together with the type of variegation which is observed in each of these plants following somatic loss of one or the other ring chromosome, respectively. + indicates normal, non-mutant tissue.

COMBINATIONS OF RING CHROMOSOMES	CHARACTER PRODUCED WHEN BOTH RING CHROMOSOMES ARE PRESENT	TYPES OF VARIEGATION: CHANGE IN CHARACTER OF TISSUE FOLLOWING SOMATIC LOSS OF ONE RING CHROMOSOME
1. X* plus normal R-2	+	Loss of normal R-2 ring: X tissue. Loss of X ring: + tissue.
2. brown *II* plus brown *III*	Plant totally brown	Loss of brown *II* ring: No change in tissue character. Loss of brown *III* ring: No change in tissue character.
3. brown *II* plus pink	+	Loss of brown *II* ring: pink tissue. Loss of pink ring: brown tissue.
4. brown plus brown-pink *II* " *I* *III* " *I* *II* " *II* *III* " *II*	Plant totally brown	Loss of brown ring: brown-pink tissue. Loss of brown-pink ring: No change in tissue character.
5. brown plus brown-blotch-dries *II* " *I* *III* " *I* *III* " *II*	Plant totally brown	Loss of brown ring: brown-blotch-dries tissue. Loss of brown-blotch-dries ring: No change in tissue character.
6. brown *I* plus brown-pink-dries *II*	Plant totally brown	Loss of brown ring: brown-pink-dries tissue. Loss of brown-pink-dries ring: No change in tissue character.
7. pink plus brown-pink *I*	pink†	Loss of pink ring: brown-pink tissue. Loss of brown-pink ring: No change in tissue character.
8. pink plus brown-blotch-dries *I*	+	Loss of pink ring: brown-blotch-dries tissue. Loss of brown-blotch-dries ring: pink tissue.
9. brown-pink plus brown-blotch-dries *I* " *I* *I* " *II*	Plant totally brown	Loss of brown-pink ring: brown-blotch-dries tissue. Loss of brown-blotch-dries ring: brown-pink tissue.

* X represents any altered ring chromosome producing a mutant character.

† Due to lack of chlorophyll, plants that are totally pink do not survive beyond the seedling stage. The test was made by combining these two ring chromosomes with a normal ring chromosome. The character produced by the combination (column 2) and the characters indicated in column 3 were obtained from sectors of these plants which had lost the normal ring chromosome.

Combination 2 in table 6 indicates that the simple mutant brown produced by the brown *II* ring chromosome is identical with the simple mutant brown produced by the brown *III* ring chromosome. Plants with these two ring chromosomes are totally brown. No detectible alteration

occurs in the tissues following loss of either ring chromosome. Combination 3 indicates that the simple mutant brown *II* and the simple mutant pink are completely independent of one another, for the pink character is suppressed in the presence of the brown *II* ring chromosome, whereas the brown *II* character is suppressed in the presence of the pink producing ring chromosome. However, the plant is variegated for both brown and pink following mitotic losses of the pink ring or the brown ring, respectively. There were no mutant sectors with the combined character brown-pink. Combination 4 indicates that the brown in the compound mutants brown-pink *I* and brown-pink *II* are identical with the brown character produced by either the brown *II* or the brown *III* ring chromosome, for these plants are totally brown although variegated for pink, following mitotic losses of the brown *II* or the brown *III* ring chromosome, respectively. Since the character brown *II* is identical with brown *III*, the brown character in the compound mutant brown-pink *I* must be identical with the brown character in the compound mutant brown-pink *II*. Similarly, as combination 5 indicates, the brown character of the compound mutants brown-blotch-dries *I* and brown-blotch-dries *II* is identical with the simple mutant character produced by either the brown *II* or brown *III* ring chromosomes. The plants are totally brown but variegated for blotch-dries following mitotic losses of the brown *II* or brown *III* ring chromosomes, respectively. Combination 6 indicates that the simple mutant brown produced by the reduced brown *I* ring chromosome is identical with the brown produced by the ring chromosome giving the compound mutant brown-pink-dries *II*. (This latter compound mutant has not been mentioned previously in this paper.) The homology of the simple mutant pink with the pink produced by the ring chromosome giving the compound mutant brown-pink *I* is shown by combination 7. However, the pink ring covers the brown part of the compound mutant produced by the altered ring chromosome giving brown-pink *I*. Combination 8 shows that the simple mutant pink and the compound mutant brown-blotch-dries *I* have no overlapping effects. The pink ring suppresses the brown-blotch-dries *I* character. Likewise, the brown-blotch-dries *I* ring chromosome suppresses the pink character produced by the pink ring. The combination of the compound mutants brown-pink *I* with either brown-blotch-dries *I* or *II* indicates that the brown character is identical in both compound mutants, for the plants are totally brown. However, the pink character produced by the brown-pink *I* ring is suppressed by the brown-blotch-dries rings, and the blotch-dries character produced by the latter ring chromosomes is suppressed by the brown-pink *I* ring chromosome.

From the combinations given in table 6, it may be seen that the brown mutant produced by an altered ring chromosome is the same whether it

appears as a simple mutant or in combination with other mutants. A similar conclusion may be drawn regarding the pink mutant, although there is less experimental evidence for this on the basis of the combinations given in table 6. It is quite obvious from these combinations that brown, pink, and blotch-dries are independent mutants and that the compound mutants result from combinations of these simple mutants. As stated earlier one may deduce the order of the segments in the R-2 ring chromosome which are responsible for the mutant effects. This order, as stated previously, was: pink followed by brown followed by blotch-dries. The experimental evidence given in table 6 supports this interpretation.

From these tests it may be concluded that the compound mutants are produced by combinations of two (or more) of the observed simple mutants and that the mutants giving the same phenotypic appearances, although arising independently of one another, are identical.

To discuss adequately the characters which are produced in plants carrying the deficient rod chromosomes following combinations of the various altered ring chromosomes would require considerable space and cannot be undertaken within the limits of this paper. However, the results of combining an altered ring chromosome with normal, non-deficient rod chromosomes 5 should be mentioned. If a plant possesses a normal rod chromosome 5 carrying *Bm* and likewise any one of the various altered ring chromosomes, the characters which these ring chromosomes would produce if deficient chromosomes 5 were present now do not appear. The plants are normal. However, when the ring chromosome is returned to plants carrying the deficient rod chromosomes in a successive plant generation, the character produced by the ring chromosome again appears. The characters produced by the ring chromosomes are thus completely recessive to "dominant alleles" which are present in segment 1 to 9 of the normal, non-deficient rod chromosome 5. This is not true, however, if a brown producing ring is combined with normal rod chromosomes 5 carrying the recessive mutant *bm*. All the ring chromosomes which give rise to the brown character (simple or compound mutants) in plants with the deficient rod chromosomes have been placed in plants possessing normal rod chromosomes 5 carrying *bm*. In every case, the plants are totally brown. In these plants, only the brown character appears. The pink, pink-dries, blotch-dries, or light green characters are suppressed by this rod chromosome 5. From these combinations it is strikingly evident that the brown character produced by the altered ring chromosomes is homologous to the known mutant *bm* previously located within region 1 to 4 of a normal chromosome 5 (McCLINTOCK 1938). A normal chromosome 5 carrying any one of the other mutants produced by the altered ring chromosomes has not been isolated as yet.

REVIEW OF THE EVIDENCE WHICH INDICATES THAT THE CHARACTERS PRODUCED BY THE ALTERED RING CHROMOSOMES ARE CAUSED BY HOMOZYGOUS DEFICIENCIES

Throughout this paper it has been assumed that the mutant characters are caused by homozygous minute deficiencies. It has been determined by many observations that ring chromosomes may increase in size by duplications and repeat duplications of segments composing the ring, or they may become reduced in size through loss of segments from the ring. It has also been determined that whole plants or sections of a plant which possess a ring chromosome with duplicated segments are not obviously modified in phenotypic appearance. Thus, the ring chromosomes with deficiencies are the ones which would be expected to produce an alteration in the phenotypic appearance of the tissues in the plants with the deficient rod chromosomes. Deficient ring chromosomes must be produced in some cells of some of these plants. It could be objected that such homozygous deficiencies might be expected to be cell lethal, and therefore the character produced by the altered ring chromosome could be caused by some other process than deficiency. However, this has proved not to be true. It is known that cells and tissues homozygous for the four chromomere deficiency of the Df-1 rod chromosome are viable. It is also known that these cells and tissues are decidedly modified. The lignified cell walls are brown. This brown is strictly comparable in time of development in the cell wall, in color, and in behavior to light to that produced by the known mutant *bm* which had previously been located within the limits of region 1 to 4 of a normal chromosome 5. (The character of the lignified walls produced when the altered ring chromosomes brown *I*, *II*, and *III*, brown-pink *I*, *II*, etc., are present is likewise strictly comparable to the character produced by the mutant *bm*.) No chlorophyll develops in the cells homozygous for the four chromomere deficiency. Therefore, with regard to chlorophyll, these tissues are colorless. Furthermore, on exposure to sunlight, these cells die and the tissue dries. These tissues have a very reduced growth capacity. It is known that the characters brown, pink, and blotch-dries must be related to changes within the region 1 to 4 of chromosome 5—that is, within the limits of the deficiency in the Df-1 chromosome. It has also been shown that the two very reduced rings (light green-poor growth and brown-light green-poor growth) produce tissues with a decidedly reduced growth capacity. Since the latter two ring chromosomes obviously do not have enough chromatin to cover the deficiency in the Df-1 chromosome, the reduced growth could be ascribed to the presence of the two chromomere deficiency in each case. If we add together the characters produced by the changed rings—that is, brown plus pink (no

chlorophyll) plus blotch-dries plus poor growth—we arrive at the compound character brown-colorless-dries-poor growth, which is exactly the compound character exhibited by the tissues which are homozygous deficient for the four chromomeres deleted from the Df-1 chromosome. This four chromomere deficiency expresses itself as a compound mutant just as brown-pink, brown-pink-dries, and brown-blotch-dries express themselves as compound mutants. Evidence for the compound nature of these mutants has been given in this paper (see table 6). It would be difficult to arrive at a more simple explanation of all these facts than that the characters are produced as the result of homozygous deficiencies, each individual character being produced by a relatively minute deficiency of a specific locus. This will explain both the simple and the compound mutants: the simple mutants resulting from loss of one specific locus, the compound mutants resulting from loss of two or more specific loci. It will also account for the recessive expression of these characters, for it is known that plants with one normal chromosome 5 and either the Df-1 or the Df-2 chromosome 5—that is, hemizygous for regions 1 to 4 or 1 to 9, respectively—are phenotypically normal.

Notwithstanding the cytological observations of reduction in size of many of the ring chromosomes giving mutation effects, genic mutation might be suggested as an alternative to homozygous deficiencies as the cause of the mutant characters. If so, two or more genic mutations would have to occur simultaneously in closely related loci in many instances to account for the considerably greater frequency of occurrence of the compound mutations. Furthermore, the rate of such mutations in the ring chromosome would have to be considerably greater than the rate of mutation in the same region of a normal rod-shaped chromosome 5. Our knowledge of "genic" mutations would not lead us to anticipate such behavior, for we know of no cases where supposedly genic mutations behave in exactly this manner.

Likewise, one might argue that the alterations in the ring chromosomes producing mutant effects are due to changes in the relative positions of the genes in the chromatin of the ring. One must then explain what should be expected of the reduced ring chromosomes, for they are being produced in these plants following aberrant mitoses which alter the chromatin content of the ring. Again, on this basis, one would have to explain away the cytologically obvious deficiencies in nine of the 15 isolated ring chromosomes which produce mutant effects. Since tissues homozygous deficient for chromomeres 1 to 4 of chromosome 5 are known to produce a compound mutant effect which equals the sum of the simple mutant effects located within this region, it would be hazardous to consider that smaller deficiencies within this region would produce no phenotypic effect. One might

object that not all the ring chromosomes giving mutant effects were detectibly reduced in chromatin content, for in four of them no deficiency was detected and in two cases the ring chromosome was enlarged. In all four cases where no deficiency in the ring chromosome was detected, simple mutants were produced (brown *II*, brown *III*, pink, pale green). If, in each case, the phenotypic expression is due to a minute deficiency, it is probable that detection of a single minute deficiency at the meiotic prophase in maize would be extremely difficult. In the cases of the two enlarged ring chromosomes it has been shown (table 6) that the characters produced by these two ring chromosomes, brown-pink *II* and brown-blotch-dries *I*, are identical with the characters produced by the reduced rings brown-pink *I* and brown-blotch-dries *II*, respectively. The enlargement does not mean that there is no deficiency in these ring chromosomes, for an enlargement may have occurred in an aberrant mitosis subsequent to an aberrant mitosis which deleted a segment from the ring chromosome. Following this deletion, no further duplication of the remaining chromatin could restore the original loss. Neither should subsequent deletions of duplicated segments cause any change in the expression of the character produced by such a ring chromosome. It should be emphasized that this proved to be true in the case of the enlarged brown-pink *II* ring.

The above considerations are the basis of the inferential evidence that the plant characters associated with altered ring-shaped chromosomes are produced as a consequence of homozygous deficiencies. In a previous section, the chromatin constitutions of the altered ring chromosomes have been considered briefly. It was pointed out that the brown-blotch-dries *II* ring, the light-green-poor growth ring, and the brown-light green-poor growth ring were all smaller than the normal four chromomere R-1 ring. In the Df-1/Df-1 or Df-1/Df-2 plants, the cells which contain only one such ring must be homozygous deficient for some chromatin within the limits of the deficiency in the Df-1 chromosome. All three of these reduced ring chromosomes may be distinguished from one another by differences in their chromomere constitution. Therefore, they do not have exactly the same deficiencies. Furthermore, each of these three rings produces, respectively, a particular and distinguishable type of compound mutant character. It would be difficult to escape the conclusion that the obviously different total deficiencies in these three rings are the cause of the obviously different total group of mutant characters that each of these three ring chromosomes produces. If one did attempt to account for these observations on some other bases, it would be necessary to assume that each deficiency in these three cases was without a distinct phenotypic effect—which hardly seems probable, considering the very striking compound phenotypic effect that a total deficiency of all four chromomeres produces.

On the basis of the above inferential and observational evidence, it is concluded that the characters exhibited in tissues containing an altered ring chromosome are caused by homozygous deficiencies, each character being caused by a relatively minute but specific deficiency, and that the compound mutant characters are caused by the removal of two or more such segments from the genomic complement.

CONCLUSION

This paper presents only a summary of the evidence which has led to the conclusion that some phenotypic characters in maize may be produced by homozygous minute deficiencies. A complete account of all the evidence obtained from altered ring chromosomes—their origin, their gametic transmissions both male and female, their stability, the variation in phenotypic expression of the various mutants when combined with different genotypes, the cytological analysis of the chromatin constitutions of the various altered ring chromosomes, the appearance of plants with various combinations of three or more altered ring chromosomes, the rate of production of altered ring chromosomes, evidence showing that the mutants do not revert to normal, and other phenomena related to the behavior of these ring chromosomes—could not be included in this paper due to limitation of space. The evidence of particular interest will be reported in separate publications. It is hoped, however, that sufficient evidence has been included to indicate the nature of the method of attacking this problem and to indicate the type of evidence obtained. Again, due to limitation of space, an adequate discussion of the relation of homozygous deficiencies giving mutant effects in maize to similar phenomena in other organisms cannot be undertaken here. It should be pointed out, however, that the number of analyzed cases is very limited and is best represented by the yellow mutant in Drosophila (EPHRUSSI 1934; STERN 1935; MULLER 1935; DEMEREC 1934, 1936; DEMEREC and HOOVER 1936; KALISS 1939).

In conclusion, the author wishes to point out the possible usefulness of the evidence presented in this paper in formulating a prediction as to the nature, the location, and the crossover values to be expected of mutants not as yet obtained in the normal complement of maize. The ring mutants pink, brown, blotch-dries, and pale green are readily transmitted through the pollen, although the relative efficiency in competition with grains carrying an unmodified complement could not be determined. It is possible, however, that through natural causes or by X-ray or ultra-violet radiation, these mutants would be produced in a normal rod-chromosome 5. Pale green and brown should produce viable plants, but pink and blotch-dries would result in plants which die in the seedling stages. A mutant (brown mid-rib, *bm*, JORGENSON 1931), allelic and indistinguishable from the

brown produced by the ring chromosome mutants, has been isolated. Although it may not be concluded from observational evidence that the *bm* mutant in the normal rod-chromosome 5 is caused by a homozygous minute deficiency, it may be stated, however, that a deficiency of the locus of the dominant allele of *bm* will reproduce in all details the phenotypic expression of *bm*. Pink, blotch-dries, and pale green remain to be isolated. Following isolation, pink and blotch-dries should prove to be very closely linked to the known mutant *bm*, whereas pale green should show a small amount of crossing-over.

SUMMARY

It is the purpose of this paper to show that viable mutants in maize may be produced by homozygous minute deficiencies. The aberrant mitotic behavior of ring-shaped chromosomes has been the method of obtaining a large number of such deficiencies. These deficiencies are located within the limits of a relatively small segment of the genomic complement composed of the proximal 9 chromomeres of the short arm of chromosome 5.

Mutants arise following changes in the chromatin constitution of the ring-shaped chromosomes. These changes are produced following aberrant behavior of the ring-shaped chromosome in some of the mitotic divisions. The types of mutants which are produced by the altered ring-shaped chromosomes are simple, composed of a single recognizable character or compound, composed of two or more of the characters recognizable as simple mutants. In a number of cases it has been possible to isolate the altered ring-shaped chromosomes which produce the simple or compound mutants.

Through appropriate tests, it has been proven that the compound mutants are the products of two or more of the simple mutants. One group of mutants has been located within the limits of the proximal 4 chromomeres of the short arm of chromosome 5. The second group of mutants has been located within the limits of the next five chromomeres.

Evidence is presented which leads to the conclusion that each mutant character, whether appearing as a simple mutant or in combination with other mutants, is produced by a homozygous minute deficiency, each mutant character being associated with loss of a particular minute segment. The simple mutants are associated with loss of one such segment; the compound mutants are associated with loss of two or more such segments.

LITERATURE CITED

EPHRUSSI, B., 1934 The absence of autonomy in the development of the effects of certain deficiencies in *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **20**: 420–422.

DEMEREC, M., 1934 Biological action of small deficiencies of X chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **20**: 354–359.

1936 Frequency of "cell-lethals" among lethals obtained at random in the X chromosome of *Drosophila melanogaster*. Proc. Nat. Acad. Sci. **22**: 350–354.

DEMEREC, M., and M. E. HOOVER, 1936 Three related X chromosome deficiencies in Drosophila. J. Hered. **27**: 206–212.

JORGENSON, L. R., 1931 Brown midrib in maize and its linkage relations. J. Amer. Soc. Agron. **23**: 549–557.

KALISS, N., 1939 The effect on development of a lethal deficiency in *Drosophila melanogaster:* with a description of the normal embryo at the time of hatching. Genetics **24**: 244–270.

MCCLINTOCK, B., 1933 The association of non-homologous parts of chromosomes in the mid-prophase of meiosis in *Zea mays*. Z. Zellf. mik. Anat. **19**: 191–237.

1938 The production of homozygous deficient tissues with mutant characteristics by means of the aberrant mitotic behavior of ring-shaped chromosomes. Genetics **23**: 315–376.

MULLER, H. J., 1935 A viable two-gene deficiency. J. Hered. **26**: 469–478.

STERN, C., 1935 The effect of yellow-scute deficiency on somatic cells of Drosophila. Proc. Nat. Acad. Sci. **21**: 374–379.

STINO, K. R., 1940 Inheritance in *Nicotiana tabacum* XV. Carmine-white variegation. J. Hered. **31**: 19–24.

BIOLOGICAL ABSTRACTS

Now published in FIVE MONTHLY SECTIONS, in addition to the present complete form.

One or more of these sections should appeal to every scientist working in Biology.

ABSTRACTS OF GENERAL BIOLOGY will include General Biology, Biography-History, Bibliography, Evolution, Cytology, Genetics, Biometry and Ecology. Priced at $4.

ABSTRACTS OF EXPERIMENTAL ANIMAL BIOLOGY will include Animal Physiology, Nutrition, Pharmacology, Pathology, Anatomy, Embryology and Animal Production. Priced at $8.

ABSTRACTS OF MICROBIOLOGY AND PARASITOLOGY will include Immunology, Bacteriology, Viruses, Parasitology, Protozoology and Helminthology. Priced at $5.

ABSTRACTS OF PLANT SCIENCES will include Phytopathology, Plant Physiology, Plant Anatomy, Paleobotany, Systematic Botany, Agronomy, Horticulture, Forestry, Pharmacognosy, Pharmaceutical Botany and Ecology. Priced at $6.

ABSTRACTS OF ANIMAL SCIENCES will include Paleozoology and Helminthology, Systematic Zoology and Economic Entomology. Priced at $5.

Subscribers to one or more of these sections will receive the Indexes to the whole of BIOLOGICAL ABSTRACTS. (Foreign subscribers add 50 cents per section for postage.)

Send for a sample copy!

BIOLOGICAL ABSTRACTS, University of Pennsylvania, Philadelphia, Pa.

Stain Technology

A Journal for Microtechnic

The latest developments in staining and general microscopy are presented in two different ways:

About 30-40 original articles each year.

Abstracts of articles appearing elsewhere, so worded that the technic may be followed without consulting the original paper.

Issued quarterly $2.25 a year

Biological Stains

By H. J. CONN, Chairman, Biological Stain Commission

A laboratory handbook on the nature and uses of dyes employed in microtechnic. About 165 dyes are discussed and over 75 of the most important staining procedures are given.

Cloth, 5½ by 8¼ in. 276 pages

$3.00 plus postage; or $2.75 postpaid, if cash accompanies order.

For sale by

BOOK SERVICE OF BIOLOGICAL STAIN COMMISSION

Lock Box 299, Geneva, N.Y.

New Publications Received

COLIN, EDWARD C. *Elements of Genetics.* Pp. xii+386. Figs. 47. The Blakiston Co. Philadelphia, 1941. $3.

ELLINGER, FRIEDRICH. *The biologic fundamentals of radiation therapy.* English translation by Reuben Gross. Pp. xvi+360. Figs. 79. Nordeman Publishing Co. New York. $5.00.

FELT, EPHRAIM PORTER. Pruning trees and shrubs. Pp. 237. Fig. 94. Orange Judd Pub. Co. New York, 1941. $2.00.

FISCHER, HELEN FIELD AND GRETCHEN FISCHER HARSHBARGER. *The flower family album.* Pp. 130. Full p. illustrations, 62. University of Minnesota Press. Minneapolis. $2.50.

POOL, RAYMOND J. *Flowers and flowering plants.* Second Edition. Pp. xxiii+428. Fig. 210. McGraw-Hill Book Co. New York, 1941. $3.50.

SHULL, A. FRANKLIN, GEORGE R. LA RUE, ALEXANDER G. RUTHVEN. *Principles of animal biology.* 5th Ed. Pp. xiv+417. Fig. 298. McGraw-Hill Company, Inc. New York. 1941. $3.50.

- **ANATOMY**
- **ANTHROPOLOGY**
- **MORPHOLOGY**
- **NEUROLOGY**
- **NUTRITION**
- **PHYSIOLOGY**
- **ZOOLOGY**

- Journals of The Wistar Institute of Anatomy and Biology merit your careful examination.
- Contributed to and edited by outstanding scientists in the respective fields.

Authoritative! ***Informative!***

Invaluable to the scientist and research worker!

For further information, address

The Wistar Institute of Anatomy and Biology
Woodland Avenue and Thirty-sixth Street
Philadelphia, Pa.

ECOLOGICAL MONOGRAPHS

(Issued under the auspices of the Ecological Society of America)

First issue published December, 1930

Managing Editors: A. S. PEARSE and C. F. KORSTIAN, *Duke University*

A quarterly journal devoted to the publication of original researches of ecological interest from the entire field of biological science. The journal will work in close cooperation with *Ecology* and will undertake the publication of papers of from 25 to 100 printed pages in length while *Ecology* will continue to specialize on papers of about 20 printed pages or less.

The board of editors will be glad to consider thoroughly scientific manuscripts which deal with any aspect of ecological investigation broadly interpreted and including community studies, ecological physiology, phenology, oceanography, biogeography, and ecological data from such practical fields as horticulture, agrioecology, economic entomology, forestry and fisheries, but will not include papers dealing primarily with economic problems.

Published in March, June, September, and December

SUBSCRIPTION: $6.00

THE DUKE UNIVERSITY PRESS
DURHAM, N.C.

Statistical Studies in Genetics and Human Inheritance

ANNALS OF EUGENICS

will continue, until further notice, to be published, in quarterly parts, without reduction in size or increase in price.

Vol. 10, No. 4, will contain articles

by

FRANK YATES — W. J. B. RIDDELL — C. H. N. JACKSON
JULIA BELL — M. G. KENDALL — K. MATHER & U. PHILIP
J. B. S. HALDANE — R. A. FISHER

REVIEWS

Subscription, in advance, 50/-per volume.

Four quarterly parts obtainable separately at 15/- each from Galton Laboratory, c/o Rothamsted Experimental Station, Harpenden, Herts.

JOURNAL *of* GENETICS

Edited by

R. C. PUNNETT

Founded in 1910, the Journal of Genetics is the only British periodical devoted to the publication of original research in Heredity and Variation. Up to December, 1935, thirty-one volumes have been completed. The illustrations form a feature of the series, for, in addition to numerous text figures and diagrams, the twenty-nine volumes contain 667 plates, of which 138 are in colors.

Many of the papers published deal with animals and plants of high economic value, and are therefore of interest to those concerned with agriculture and horticulture.

A full index of Vols. XIII-XXIV was issued with the last part of Vol. XXIV.

The Journal of Genetics is published in parts, of which three form a volume. Approximately two volumes are issued annually.

The Cambridge University Press has appointed The University of Chicago Press agent for the sale of the Journal of Genetics in the United States of America, and has authorized the following prices: Annual subscription $10.00 net; single copies $3.50 net each. The parts are sent post free to subscribers as issued.

Inquiry as to back numbers should be made to The Cambridge University Press. Separate parts and volumes are still available in some cases.

INFORMATION FOR CONTRIBUTORS

Contributions to GENETICS may be in the field of genetics proper, of cytology, taxonomy, embryology, physiology, biometry, or mathematics, if of sufficient importance and of such a character as to be of primary interest to the geneticist. For the present, the length of manuscripts will be limited to twenty-five printed pages (about twelve thousand words) except by special vote of the Editorial Board. Tabular matter in excess of one-fourth of the manuscript can not be printed, unless of particular importance, but will be kept on file for reference on request provided two copies are furnished by the author. Excess pages will be printed if paid for by the author (about six dollars a page).

Manuscripts are printed, ordinarily, in the order of their receipt. They may be printed out of turn provided the entire cost is paid by the author. Such material will be added to the current number and will not delay the publication of any other article.

Contributors are requested to use care in the preparation of manuscripts. Carbon copies can not be considered. All references to literature should cite the name of the author, followed by the year of publication, the papers so referred to being collected into a list of "LITERATURE CITED" at the end of the article. In this list care should be taken to give the titles in full, and to indicate accurately, in Arabic numerals, the volume number, the first and last pages, and the date of publication of each paper if published in a periodical, and the number of pages, place and date of publication, and the name of publisher, of each independent publication. The arrangement of this list should be alphabetical by author and chronological under each author. Titles of publications are abbreviated according to the *World list of scientific periodicals*, Oxford University Press, London and New York, 1925.

Each manuscript should include a summary of the evidence and of the conclusions. Factor symbols should be separated so that they can be properly identified and underlined for italics. Gene symbols with sub- or superscript letters should be avoided except in long allelic series. Most typewriters do not distinguish between the letter l and the figure 1 or the hyphen and the dash. Such distinctions should be made wherever there is a possibility of confusion.

Footnotes should be avoided wherever possible. Usually, they can be enclosed in parentheses and inserted after the sentences to which they apply. If used in the text they should be numbered consecutively in a single series and designated by Arabic superscript numerals. Footnotes to tables should be marked with an asterisk, dagger, or other symbol so as not to be confused with the figures in the tables.

Illustrations should be referred to as figures wherever possible. Plates are reserved for illustrations that require paper inserts or for collections of small figures that can not be designated conveniently as separate figures. Text figures should be clearly identified but not numbered in the illustration. Figures included in plates should be distinguished by letters rather than by numbers. All figures and plates are reduced to a maximum of 4⅜ inches in width and 6 inches in height.

Legends for figures and plates should be typewritten separately from the illustrations for the reason that the type is set by the printer and the illustrations are made by the engraver. Mistakes are likely to occur if the separation is not made by the author.

Galley proofs and, whenever there is time, page proofs will be sent. Authors should leave forwarding directions whenever they are to be away from the address sent with the manuscript. Ordinarily page proofs can not be sent out of the country. Both proofs must be returned promptly and no extensive change may be made in page proofs, which is not compensated for within the same paragraph, or in an adjacent paragraph on the same page. Changes from copy will be charged to the author.

GENETICS furnishes 75 reprints, without covers, free. Covers and additional reprints may be secured at actual cost of manufacture provided these are ordered when the corrected galley proofs are returned.

Manuscripts and all editorial correspondence should be addressed to the Editor of GENETICS, 704 Schermerhorn Hall, Columbia University, New York.

GENETICS Vol. 26 No. 5 SEPTEMBER, 1941

CONTENTS OF PRECEDING ISSUES

NOVEMBER, 1940

RAFFEL, DANIEL, and MULLER, H. J., Position effect and gene devisibility considered in connection with three strikingly similar scute mutations.

GREEN, M. M., and OLIVER, C. P., The action of certain mutants upon the penetrance of heterozygous vestigial wing in *Drosophila melanogaster*.

COOPER, D. C., and BRINK, R. A., Somatoplastic sterility as a cause of seed failure after interspecific hybridization.

DEMEREC, M., Genetic behavior of euchromatic segments inserted into heterochromatin.

SUTTON, EILEEN, Terminal deficiencies in the X chromosome of *Drosophila melanogaster*.

CLELAND, RALPH E., Analysis of wild American races of Oenothera (Onagra).

BRODY, GEORGE, The time of effect of some second chromosome lethals in *Drosophila melanogaster*.

Index to Volume 25.

JANUARY, 1941

Portrait of RAYMOND PEARL.

HELFER, R. G., A comparison of X-ray induced and naturally occurring chromosomal variations in *Drosophila pseudoobscura*.

DOBZHANSKY, TH., and WRIGHT, SEWALL, Genetics of natural populations. V. Relations between mutation rate and accumulation of lethals in populations of *Drosophila pseudoobscura*.

NEEL, JAMES V., Studies on the interaction of mutations affecting the chaetae of *Drosophila melanogaster*. I. The interaction of hairy, polychaetoid, and hairy wing.

GRIFFITHS, RAYMOND B., Triploidy (and haploidy) in the newt, *Triturus viridescens*, induced by refrigeration of fertilized eggs.

NICHOLS, CHARLES, Spontaneous chromosome aberrations in Allium.

HUSKINS, C. L., and NEWCOMBE, H. B., An analysis of chiasma pairs showing chromatid interference in *Trillium erectum* L.

NEWCOMBE, H. B., Chiasma interference in *Trillium erectum*.

Abstracts of papers presented at the 1940 meetings of the Genetics Society of America.

MARCH, 1941

CASTLE, W. E., Influence of certain color mutations on body size in mice, rats, and rabbits.

GREEN, EARL L., Genetic and non-genetic factors which influence the type of the skeleton in an inbred strain of mice.

BURKS, BARBARA S., and WYANDT, HELEN, Oval blood cells in human subjects tested for linkage with taste for PTC, middigital hair, hair color, A-B agglutinogens, and sex.

MCCLINTOCK, BARBARA, The stability of broken ends of chromosomes in *Zea mays*.

MAY, 1941

CLARK, FRANK H., Correlation and body proportions in mature mice of the genus Peromyscus.

GREENLEAF, WALTER H., Sterile and fertile amphidiploids: Their possible relation to the origin of *Nicotiana tabacum*.

STEINBERG, ARTHUR G., A reconsideration of the mode of development of the Bar eye of *Drosophila melanogaster*.

BRASTED, ADAIR, An analysis of the expression of the mutant "engrailed" in *Drosophila melanogaster*.

LESLEY, MARGARET MANN, and J. W. LESLEY, Parthenocarpy in a tomato deficient for a part of a chromosome and in its aneuploid progeny.

JULY, 1941

LINDSTROM, E. W., Genetic stability of haploid, diploid, and tetraploid genotypes in the tomato.

BLAIR, ALBERT P., Variation, isolating mechanisms, and hybridization in certain toads.

SAX, KARL, The behavior of X-ray induced chromosomal aberrations in Allium root tip cells.

LANDAUER, WALTER, A semi-lethal mutation in fowl affecting length of the upper beak and of the long bones.

STEINBERG, ARTHUR G., Studies on modifier of Bar. A mutant which affects the expression of the Bar mutation of *Drosophila melanogaster*.

ANDERSON, E. G., Translocations in maize involving the short arm of chromosome I.

BURNHAM, C. R., Cytogenetic studies of a case of pollen abortion in maize.

SINGLE NUMBERS $1.25 ANNUAL SUBSCRIPTION $6.00

obtainable from

BROOKLYN BOTANIC GARDEN, BROOKLYN, NEW YORK